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RELATION OF PARALYTIC SHELL-FISH POISON TO CERTAIN PLANKTON ORGANISMS OF THE GENUS GONYAULAX

HERMANN SOMMER, PH.D.

W. F. WHEDON, M.A.

C. A. KOFOID, PH.D.

AND

R. STOHLER, PH.D.

SAN FRANCISCO

INTRODUCTION

BY DR. SOMMER

Many theories have been proposed for the explanation of the occurrence of poison in shell-fish. The more likely ones were enumerated in the first paper of this series.[1] As these studies during the past ten years progressed,[2] most of the theories could be abandoned, as it became more and more apparent that the agent responsible for the toxicity was contained in the ocean water and approached the shell-fish beds, more or less periodically, from offshore. Since the hypothesis that the toxic agent was an organism pathogenic for mussels, or the assumption that the toxicity was due to an actual disease, did not receive any support, the search for the causative factor narrowed down to a dissolved or particulate substance in the water which the mussels were taking up. It was obvious that the food of the shell-fish, i. e., the diatoms and dinoflagellates, were one possibility.

In 1928 it was mentioned that the "food (certain dinoflagellates) may be one of the factors responsible."[1] Stohler, who completed a short histologic study of poisonous and normal mussels in 1930, seemed to be able to distinguish normal from poisonous mussels on the basis of their stomach contents; the latter seemed to contain some "diatoms" which were absent in the former. His paper is included in the present

From the George Williams Hooper Foundation, University of California.

1. Meyer, K. F.; Sommer, H., and Schoenholz, P.: J. Prev. Med. **2**:365, 1928.

2. (*a*) Meyer, K. F.: Am. J. Pub. Health **21**:762, 1931. (*b*) Sommer, H., and Meyer, K. F.: California & West. Med. **42**:423, 1935; (*c*) Arch. Path., this issue, p. 560.

series. From the figures it may be seen that some of the "diatoms" are unquestionably identical with the organisms which were later called Gonyaulax catenella.[3] However, Stohler's conclusions could not be substantiated by further studies in 1930 and 1931; the general microscopic picture in poisonous and normal mussels is very much the same at the same time of the year if the animals have been fixed at the same stage of the digestive process. A more detailed and quantitative investigation of all organisms which mussels ingest was needed.

These studies of the food of mussels were begun in June 1932, concurrent with frequent tests for poison in mussels from the same locality.[2c] At the same time, the finding of an identical poison in the sand-crab,[4] another feeder on plankton, strengthened the belief that the food of the shell-fish would be found to be the source of the poison. Two accidental circumstances favored the progress of these studies. At the beginning of the detailed counts of the organisms in plankton, during a period of strong toxicity in 1932, a new species of dinoflagellates (Gonyaulax catenella) was discovered in large numbers,[5] which aroused immediate suspicion, and toward the end of the period herein detailed these same organisms increased to such numbers that the influence of all other species could safely be disregarded and the connection between plankton and shell-fish poison proved with a few conclusive experiments.

Apart from the identification of the species of the plankton and the correlation of their numbers with the toxicity, attempts were started in 1930 to demonstrate the poison in the plankton directly. These trials were unsuccessful until 1933, after the chemical behavior of the poison had become better known.[5] Further progress in the methods of extraction and the fortunate occurrence of large numbers of toxiphoric organisms in June 1935 led to isolation of the poison in relatively large amounts from plankton. The quantitative relations and the mechanism by which the poison from the plankton is stored by the mussels were further elucidated by feeding experiments.

HISTOLOGIC OBSERVATIONS ON TOXIC AND NONTOXIC MYTILUS CALIFORNIANUS CONRAD

By Dr. Stohler

From the beginning, the problem of mussel poisoning appeared to be complex. For this reason, it was approached from as many different sides as possible. Thus, many results were obtained which may have

3. Whedon, W. F., and Kofoid, C. A.: Univ. California Publ., Zool. **41**:25. 1936.

4. Sommer, H.: Science **76**:574, 1932.

5. Mueller, H.: J. Pharmacol. & Exper. Therap. **53**:67, 1935.

no connection with the cause of the toxicity of Mytilus californianus Conrad but which are of interest and are therefore worth reporting. Possibly such results are those gained in the course of the histologic investigation of toxic and nontoxic mussels. Among other findings, these have stimulated further research. Their final evaluation, therefore, must be withheld until the newer investigations are concluded. The intention in the present communication is merely to record the results for use at a later date.

Field [6] and List [7] have published excellent papers on the histology of Mytilus edulis Linnaeus and several other Mytilides. However, no publication of this kind has been found in the literature dealing with Mytilus californianus Conrad. The normal histology of this mollusk should be worked out in every detail to serve as a basis for any comparison in any investigation on the abnormal appearance of the organs of the mollusk. In the relatively short time since the first catastrophe in the year 1927 it has not been possible to determine what should be considered as normal in mussels. Even less possible—and not desired—was the working out of the normal histology, since circumstances would not permit such work, which would necessarily extend over several years. In the first place, some specimens of Mytilus californianus Conrad which from the test of their toxic action on mice were considered nontoxic had to serve as normal for comparison. It was also necessary to refer to the foregoing citations. Some apparently essential differences in the digestive gland and also in some other organs of Mytilus californianus Conrad in comparison with Mytilus edulis Linnaeus and other Mytilides make a comprehensive and careful study of the histology of the aforementioned mollusk most desirable.

The material for the present paper was collected on many field trips along the Pacific Coast. The region covered extends over 600 miles of coast, measured in air-line, i. e., from La Jolla (near San Diego, San Diego County, in southern California) to north of Salmon Creek (north of Bodega Bay, Sonoma County). The animals were fixed by plunging the usually narcotized (according to Lo Bianco) and still living specimens into the fixing fluid, after the musculus adductor anterior had been cut through. Twenty-four hours later the mussels were removed from their shells and replaced in the fixing fluid if Bouin's fluid [7a] was used. At least three specimens were fixed each time, namely, one in Zenker's fluid, one in Bouin's fluid and one in a mixture of solution of formaldehyde U. S. P. and sea water. In the last case treatment with mercuric chloride solution in sea water followed. Fleming's fluid was also tried. Zenker's fluid, however, proved to be far superior for the purposes of this study in all cases. The embedding was carried out in the usual manner in paraffin.

6. Field, I. A.: Bull. U. S. Bur. Fisheries **38**:127, 1921-1922.

7. List, T.: Die Mytiliden: Fauna und Flora des Golfes von Neapal, Monograph 27, Berlin, Friedlaender & Sohn, 1902.

7a. The formula for Bouin's fluid is: trinitrophenol (saturated aqueous solution, 15 cc.; solution of formaldehyde U. S. P., 5 cc., and glacial acetic acid, 1 cc.).

After many trials 6 microns was determined to be the most favorable thickness for sectioning. Of the staining methods tested, including various hemalum mixtures with or without an eosin counterstain, and hematoxylin mixtures with or without different counterstains, the Ehrlich acid-hematoxylin mixture with an eosin counterstain and the modified Weigert iron-hematoxylin method gave the best results. Therefore these two methods were subsequently applied exclusively. A number of the stained sections were mounted in Canada balsam; an equal number, in hyrax.[8] The latter proved to be of great value in examinations of stomach contents, owing to its extremely high index of refraction (1.822). It cannot be recommended, however, for every purpose, since it has been found to change the eosin to a dull red and, to a lesser degree, to attack the Ehrlich hematoxylin. In certain organs shrinkage occurs while the hyrax is drying. But when these disadvantages are not present the clearness and depth of the pictures are remarkable.

Wolff [9] was able to demonstrate that the poison of Mytilus edulis L. is localized mainly in the "liver" (the digestive gland). This organ was therefore investigated above all with great care. In the sections stained with Ehrlich's hematoxylin, a yellow substance could be observed in the secondary hepatic tubules. This substance was always present whether the mussels were poisonous or not. Only in starved specimens was it absent. Besides this difference, which could be shown to be due to the degree of nourishment of the mussel, no differences could be observed in all digestive glands of toxic and nontoxic mussels which were investigated.

Among many others, the opinion was expressed that the toxicity of the mussels was closely connected with their sex cycle. Although this statement could not be questioned—if not disproved—by other means (for instance, a comparison of spawning times at the different localities with the extension and restriction of the range of the poisonous Mytilus), special attention was given to this point in the histologic examination. Ripe or ripening sex products were observed in the mantles of both toxic and nontoxic mussels. Empty or almost empty mantles were found also in both toxic and normal specimens. No distinct difference could be established here. This statement applies to males and females alike. However, it was possible to prove that the sexes in Mytilus californianus Conrad are separate.[10]

The contents of one organ seemed to give a lead. In the stomach of the toxic mussel certain plankton organisms were found in considerable numbers, which were observed either not at all or only in very small amounts in the stomach of the nontoxic mussel. These organisms were probably diatoms; however, the species has not been identifiable so far. In mussels that were almost nontoxic, only a few small fragments of

8. Hanna, G. D.: Science **65**:41 and 575, 1927.
9. Wolff, M.: Virchows Arch. f. path. Anat. **103**:187, 1886.
10. Stohler, R.: Zool. Anz. **90**:263, 1930.

these organisms were found, as can be seen in figure 1 *A*. In toxic animals the field of vision was almost entirely filled with these planktonts (fig. 1 *B* and *C*). This statement is brought out still more clearly in table 1. In specimens of low toxicity a small number of these organisms were sometimes visible; sometimes none at all were present (fig. 1 *D*).

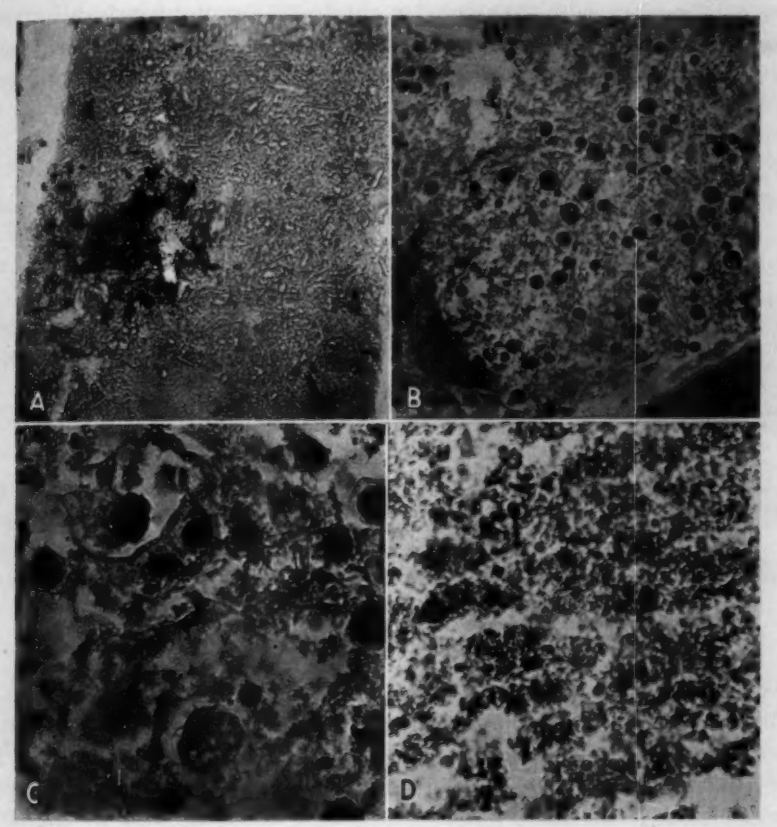

Fig. 1.—*A*, stomach contents of Mytilus californianus Conrad Green Canyon, near Montara, San Mateo County, Calif., Oct. 15, 1929; Weigert's iron-hematoxylin method, modified; hyrax (prep. 177:37); × 442. The toxicity was 1 (on an arbitrary scale). The content of the stomach consists almost entirely of small planktonts without shell, but there are some fragments of small shells present. *B*, stomach content of Mytilus californianus Conrad, Miramar, San Mateo County, July 11, 1929; Weigert's iron-hematoxylin method, modified; Canada balsam (prep. 150:15); × 110. The toxicity was 64. The stomach is filled almost entirely with suspected planktonts. *C*, a part of the previous picture at higher magnification; × 442. *D*, stomach content of Mytilus californianus Conrad, Cliff House, San Francisco, July 12, 1929; Weigert's iron-hematoxylin method, modified; hyrax (prep. 162:22); × 442. The toxicity was 8.

The hypothesis, then, is that the presence in varying degrees of the organisms which are here called diatoms need not be a coincidence. What connection these planktonts have with the toxicity of the mussels cannot and should not be decided here. The possibility exists that the organisms are only an indicator of the general external conditions, which themselves may influence in some manner the appearance of poison in the mussels. It is also possible that in the metabolism of the mussel the observed planktonts give rise to a substance which in further metabolic processes becomes the mussel poison. An additional possibility is that the plankton organisms already contain a toxic substance,

TABLE 1.—*Relation of Suspected Organisms to Toxicity*

Locality on Coast of California	Date	Toxicity*	Number of Suspected Diatoms	Number of Other Organisms	Number of Spores†
	1928				
Carmel Bay	Oct. 27	0	0	192	0
	1929				
Carmel Bay	April 17	0	6	71	0
Salada Beach	Sept. 2	1	6	137	0
Green Canyon	Oct. 15	1	2	272	0
Dillons Beach	July 13	4	6	11	0
Cliff House, San Francisco	July 12	8	18	32	0
Salmon Creek	July 13	8	28	0	286
Salada Beach	July 29	8	31	58	0
Moss Beach	July 11	16	27	36	0
Point Reyes	July 13	16	53	67	0
	1930				
Salmon Creek	Jan. 24	16	29	193	0
	1929				
Miramar	July 11	64	96	0	0
Salada Beach	July 12	120	40	0	880
	1927				
Point Reyes	.....	Toxic	38	110	0
Long Beach	.....	Nontoxic	32	70	0

* In these tests an arbitrary scale of grading toxicity was used.
† These were apparently coming out of suspected diatoms.

which is assimilated by Mytilus and stored in the digestive gland. Aside from variations and complications of those mentioned, a fourth possibility exists, that the presence of these organisms in toxic mussels is after all only a coincidence, however remarkable. Which of these possibilities is the most probable will have to be decided by the new investigations stimulated by these findings. Until then all conclusions should be avoided.

RELATION OF PLANKTON COUNTS TO PARALYTIC SHELL-FISH POISON

BY MR. WHEDON and DRS. KOFOID and SOMMER

The purpose in this section is to describe the methods employed in the studies of plankton and to discuss the relation of certain species of dinoflagellates to the occurrence of mussel poison in the region of San Francisco.

EXPERIMENTAL OBSERVATIONS

An extensive study of the plankton was begun during June 1932, which was continued until July 1935. A preliminary investigation had been conducted during the summer of 1931 to determine the specific plankton forms used as food by the mussels, in view of the diversity of opinion on this subject among the various marine investigators. Evidence to support the contention that mollusks possess the power to discriminate between suitable and unsuitable types of food had been presented by Erman,[11] Thiele,[12] Lotsy [13] and Field.[6] List [7] took exception to this theory and made the observation that all bodies in the sea water not exceeding a certain size are taken into the mouth. The results of the preliminary investigation in 1931 revealed that the mussel Mytilus californianus Conrad exercises selectivity in regard to species of dinoflagellates and also chooses forms which do not exceed specific dimensions. Diatoms, of course, form a portion of the food supply, but they, as will be shown later, may be excluded from consideration as a factor in the occurrence of the poison.

In addition to establishing the plankton forms used as food, it was necessary to determine the approximate amount of water an average-sized mussel would filter during the course of one feeding. This was done by comparing the number of dinoflagellates in the stomach contents of one mussel with the number in varying quantities of water. The results, with evidence derived from a similar study of a closely related mollusk, Mytilus edulis, by Dodgson,[14] led to the assumption that approximately 19 liters of sea water was filtered at one feeding. This quantity of water was taken as the standard for the studies of plankton which followed, more as assuring a maximal representation of species than as affording accurate estimates of the dinoflagellates and diatoms present in the catches. In counting these catches only those species of dinoflagellates were considered which had previously proved to be a part of the food supply of the mussels. The values obtained (fig. 1 *A*) represent the thecate forms observed in the samples, with one or two exceptions, as the addition of preservative almost invariably destroyed the nonthecate types. The method employed in making the counts has been described in another report to appear shortly.[15]

11. Erman: Abhandl. d. k. Akad. d. Wissensch., Phys. Kl., 1883, p. 527.

12. Thiele, J.: Ztschr. f. wissensch. Zool. **44**:239, 1886.

13. Lotsy, J. P.: Johns Hopkins Univ. Circ. **12**:104, 1893.

14. Dodgson, R. W.: Report on Mussel Purification, Ministry of Agriculture and Fisheries, London, 1928.

15. Whedon, W. F.: A Three Year Survey of the Phytoplankton in the Region of San Francisco, California, to be published.

COMMENT

The sources of probable error in investigations of this nature are many and must be given careful consideration in order to obtain a complete understanding of the findings. The destruction of the nonthecate forms may be cited as one source of possible error, but since these dinoflagellates cannot be demonstrated in the stomach contents of the mussels, their absence in the water samples is not of extreme importance. The method of collecting the water samples and the natural variability in numbers of dinoflagellates which may occur during the course of a day must be considered as possible sources of error.[16] Samples were collected and concentrated simultaneously by net filtration and sedimentation methods for a period of two and a half months in 1933 in order to determine the actual loss of plankton, especially of dinoflagellates, through the mesh of the net. The number of organisms counted in the samples concentrated by the sedimentation method was always much greater than that with net filtration.[15] The first method is more exact, but the purpose for which the counts were intended was of such nature that general trends, as shown by net filtrations, were found to be sufficiently accurate to supply the necessary data. Whenever possible, the samples were collected at high tide, since it was then that the mussels fed most actively; however, there were times when this was impossible. In a few instances samples were collected both morning and afternoon. The numbers obtained on the afternoon samples were larger on a few occasions by three or four times. The increase in each instance was due to one or two species. It is highly probable, therefore, that the sample taken at any time other than that of high tide did not present the true facts. Allen [17] has suggested that tidal currents may exert some influence on increase and decrease in plankton. These are a few of the various sources of unexpected error which may account for the variations between the poison and Gonyaulax curves shown in figure 2.

The results of three years of investigation by qualitative and quantitative methods indicate that the yearly maxima of certain species of the genus Gonyaulax occurred preceding and during each poison period. However, the major pulses of this genus did not always coincide with those of the other dinoflagellates. There were several species which appeared, usually at a later date, in numbers which greatly exceeded those of the genus Gonyaulax, but these increases were not accompanied by an increase in the toxicity of the mussels. A striking example of this was demonstrated during 1934, when the yearly maximum of

16. Allen, W. E.: Bull. Scripps Inst. Oceanog., Techn. Ser. **2**:319, 1930.

17. Allen, W. E.: Univ. California Publ., Zool. **22**:369, 1922; Bull. Scripps Inst. Oceanog., Techn. Ser. **1**:201, 1928.

Gonyaulax catenella Whedon and Kofoid,[8] 2,702 cells per liter, occurred on June 10. Later, on August 27, the maximum number of Ceratium furca Ehrenberg, 28,908 cells per liter, occurred. There was also an increase in the number of Gonyaulax at that time, with a corresponding rise in the toxicity of the mussel (fig. 2). If the Ceratium had been responsible for this increase in toxicity, the increase would have been considerably greater than it actually was.

The diatoms greatly outnumbered the dinoflagellates in all but a very few of the catches. At times during the spring months the estimated

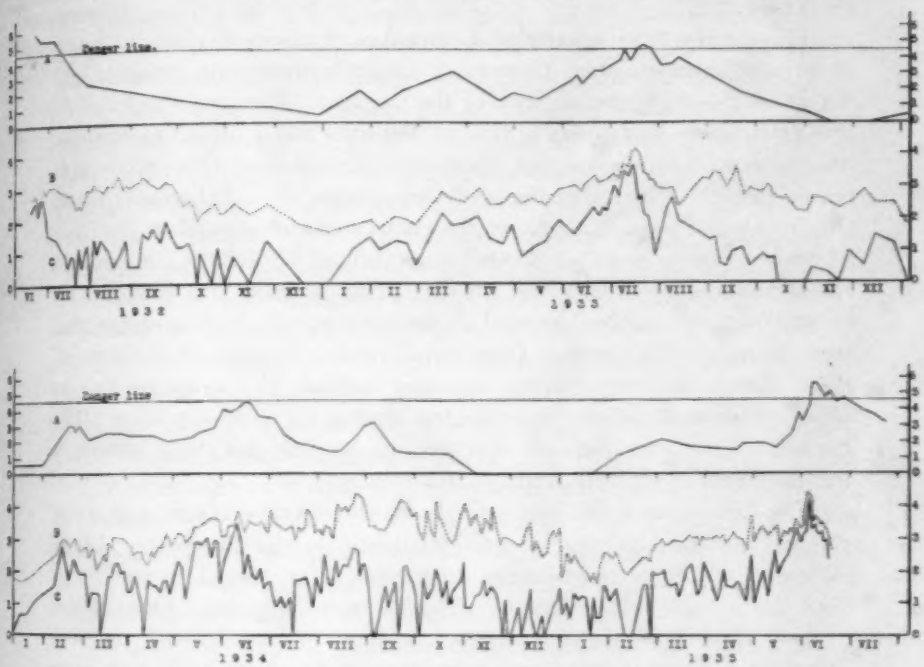

Fig. 2.—Graph showing the relation of the number of dinoflagellates to the amount of shell-fish poison in mussels: *A* represents the amount of poison; *B*, the total number of dinoflagellates; *C*, the number of organisms of the suspected species of Gonyaulax. The number of dinoflagellates and that of the units of poison are given in logarithms.

numbers reached several million cells per liter. There was no accompanying increase in the quantity of poison extracted from mussels collected at the times of these maxima in diatoms. The diatoms, therefore, may be excluded from further consideration.

The species of Gonyaulax found to be most prominently associated with increases of the poison in the San Francisco region was Gonyaulax catenella Whedon and Kofoid, although others, namely, Gonyaulax digitale Pouchet, Gonyaulax polygramma Stein, Gonyaulax spinifera

Claparede and Lachmann, and Gonyaulax triacantha Jörgensen, were also present. All of these forms demonstrated a similar change in color. Normally, the color of the cell contents is yellow-green or golden, but accompanying all increases in the poison, both major and minor, the color assumed a reddish brown or orange-brown cast in most of the individuals. Gonyaulax catenella appeared most consistently in the collections throughout the year, and its seasonal total number was greater than the combined numbers of the other species. In fact, these forms could be disregarded without materially changing the results shown in the graph.

There were other species of Gonyaulax in the collections, at times in greater numbers than Gonyaulax catenella, but their presence did not affect the degree of toxicity of the mussels. The forms included in this group were Gonyaulax acatenella Whedon and Kofoid,[3] Gonyaulax diegensis Kofoid, Gonyaulax alaskensis Kofoid and Gonyaulax unicornis Lebour. The cell contents of these forms did not change in color, thus making it possible to divide the genus into two groups on the basis of the variability in color. Collections taken at La Jolla, Calif., during the occurrence of "red water" in the spring of 1933 and examined in this laboratory contained several of the same species of Gonyaulax that were found in the samples from San Francisco. The cell contents of these forms, however, were deep red instead of orange-brown, as observed in those of the latter region during the poison periods. The records of toxicity indicate that the poison has not been definitely demonstrated in mussels from the La Jolla region.

The literature of the past years contains several accounts of similar changes in the color of the cell contents of dinoflagellates. Large increases usually occurred at the same time, accompanied by patches of "red water" and quite often by phosphorescent displays. Occurrences of both these phenomena have been reported at San Diego.[18] In both instances these outbreaks were accompanied by abnormally high mortality of bottom-dwelling marine forms. The causative organism was found to be Gonyaulax polyedra Stein, which was present in large numbers. In 1932, too late for investigation, "red water" was reported to this laboratory by a resident of Bodega Bay, Calif., but since that date no further occurrences of this type have been reported.

Other species of dinoflagellates are known to have caused "red water" and phosphorescence. The occurrence at La Jolla, Calif., in 1933[19] was caused by greatly increased numbers of Prorocentrum micans Ehrenberg. During the summer of 1934, a similar occurrence

18. Torrey, H. B.: Am. Naturalist **36**:187, 1902. Kofoid, C. A.: Univ. California Publ., Zool. **8**:187, 1911.

19. Allen, W. E.: Science **78**:12, 1933.

was noted in the vicinity of Seattle. Samples collected and sent to this laboratory by H. W. Nightingale, of the Washington state department of health, contained an abundance of Gymnodinium splendens Lebour. Another sample from the same source, which was collected during a period of excessive phosphorescence, contained an almost pure culture of Noctiluca scintillans Macartney. No records of poison tests are available for that region for the year 1934.

An occurrence of the poison in mussels along the coast of northern California and southern Oregon during September 1933 was preceded by brilliant displays of phosphorescence. Since similar displays had been observed in that region many times before, no particular significance was attached to this one. However, one death and several illnesses from eating mussels were reported to the state department of health during the following week. Investigation of the plankton from the waters of this region immediately after these reports disclosed that Gonyaulax catenella was present in most of the samples examined. The numbers were small, but the appearance of the cell contents was identical with that seen in the same form during occurrences of the poison in the vicinity of San Francisco. To all appearances Gonyaulax was disappearing at the time the examination was made.

A major outbreak of "red water" occurred at Gokasho Bay, in Toba Japan, during November and December 1933 and January and February 1934. Mr. Oda and Mr. Mikimoto, who kindly forwarded information concerning this occurrence, have stated that it was due to an influx of Gymnodinium mikimotoi n. sp. Approximately from 3,500 to 5,000 organisms were counted in 1 cc. of sea water, according to Mr. Oda. The accumulation of large masses of these organisms on the surfaces of the gills of pearl oysters and fish resulted in death of these animals. No mention was made of the presence of toxic substances.

In some respects the Japanese outbreak was similar to the one at San Diego, Calif., since extremely high mortality was observed among the marine animals in the affected areas in both instances. The death of the animals during the San Diego occurrences was attributed to the accumulation of toxic substances in the sea water following the decay of large quantities of nitrogen-bearing plankton, specifically, Gonyaulax polyedra Stein. In view of the present biologic and chemical findings, which clearly show that the poison exists within the living plankton, this earlier assumption will, no doubt, have to be revised. Quantities of shell-fish poison, much larger than have been measured in the San Francisco region, quite possibly existed in the living plankton at that time, but since tests for toxin were not made this cannot be checked. Perhaps if the numbers of Gonyaulax catenella and related species associated with it during the present investigation had been greater, the

quantities of poison extracted from the digestive glands of the mussels might have been many times larger than the tests have indicated, and the poison, possibly of sufficient potency to have caused either metabolic disturbances in the mussels or their death.

The resistance of shell-fish, as well as of many other cold-blooded invertebrate and vertebrate forms, both marine and terrestrial, to comparatively large quantities of the poison is of especial interest since warm-blooded animals are so highly sensitive to it. A thorough study of the metabolic processes of the mollusk failed to show any significant variations of the respiratory quotient in either toxic or nontoxic animals,[20] and the death rate did not increase. Proportionately larger doses of the poison had to be injected into frogs than into mice, dogs or cats to produce symptoms of mussel poisoning.[21] A salamander, Batrachia, commonly found in gardens in the vicinity of San Francisco, has been found to be at least forty-five times more resistant to the poison than the average-sized laboratory mouse used in determining the toxicity of the mussels (unpublished data by Sommer). Human beings have been known to suffer severe reactions after eating a dozen highly toxic mussels. Additional investigation of this subject should demonstrate the effects of this poison on these two groups of animals more clearly.

The color variations noted during this study are probably indicative of physiologic changes within the cell contents of the dinoflagellates, which may or may not be associated with the occurrence of the poison. Alterations in the chemical balance of the sea water, which do not have to be large to affect these microscopic forms, may explain the color changes. The rapid increase in the number of Gonyaulax catenella which preceded each period of poison in the mussels and was accompanied by color variations is possibly to be attributed to a specific stage in the life cycle of this organism. It is quite probable that the changes in color and the occurrence of the poison are associated with the luminescent properties of the genus Gonyaulax. An explanation of the cause of these changes in color and of the presence of the poison in living plankton must await further investigation.

FEEDING OF MUSSELS IN THE LABORATORY

By Dr. Sommer and Mr. Whedon

Mussels may be kept in the laboratory, in clean aerated sea water, without food, for many weeks. While the time during which the shell-fish are likely to survive varies with different lots of mussels, two months may be considered a minimal period. Experiments have been made on

20. Whedon, W. F., and Sommer, H.: Studies of the Respiratory Quotient of the Mussel Mytilus Californianus Conrad, to be published.

21. Prinzmetal, M.; Sommer, H., and Leake, C. D.: J. Pharmacol. & Exper. Therap. **46**:63, 1932.

the changes taking place during this time, in the sea water and in the weight and appearance of the organs of the mussels.

It seemed of primary importance, also, to follow the poison titer of the mollusks during these keeping experiments. It has been mentioned previously [2c] that the toxic mussels under these conditions gradually lose their potency. The rate of detoxification varies with different samples. From data on numerous tests it may be concluded that the toxicity drops to one half in about ten days, the curve having the general shape of a logarithmic function. In table 11 of that publication [2c] the data on two samples are detailed. It will be seen that the toxicity and the amount of extract obtained from a standard sample drop at somewhat irregular rates. This variability may be explained best by assuming individual differences of single mussels in the rate of detoxification. An apparent slight increase in toxicity must be interpreted on these grounds. A substantial gain in toxicity has never been observed in holding experiments with starving mussels in filtered sea water.

Since running sea water was not available for these studies, few attempts have been made to feed mussels in the laboratory. In a preliminary experiment, between Sept. 21 and 26, 1932, six mussels were kept in 1 liter of filtered sea water. Every day approximately a hundred buckets of water (750 liters) were filtered through a no. 25 net, at San Francisco Beach, and the filter residue fed to the mussels. The organisms in the plankton were counted immediately after the addition of the concentrate to the jar and twenty-four hours later. The initial count ranged from 300 to 700 dinoflagellates per cubic centimeter; at the end of every twenty-four hour period, from 4 to 26 per cent of this number were left. How much of the food had been consumed by the mussels is difficult to judge, since the animals seemed to close their shells as soon as the concentrate was added. No change in toxicity was observed during the experiment. A control sample of mussels, kept in filtered sea water, likewise showed the same poison content at the end of the test. For comparison with later data it should be mentioned that the six mussels received a total of some 70,000 Gonyaulax catenella during these six days.

The results of the tests just mentioned and other observations seemed to indicate that plankton concentrates are not well suited for the feeding of mussels. One reason for this may be that many of the organisms in the concentrated sample die off in a short time and give rise to decomposition products which are objectionable to the mussels. An experiment was therefore performed in which fresh sea water was fed at a time when sufficient plankton organisms were expected to occur. The time chosen was late in the spring of 1935, after the number of Gonyaulax catenella had increased.

Ten mussels were used which had been kept on an experiment in the laboratory since April 24, 1935. Although their history previous to the experiment is not believed to be of importance, it is outlined here. One half of the shell-fish received sodium nitrite in small amounts, while the other half were kept as controls. The original toxicity of 11.7 mg. dropped to 35 mg. in the "nitrite mussels" and to 40 mg. in the controls by May 9. The shell-fish were then combined in one jar and small amounts of chlorine added from May 14 to 18 for the purpose of sterilization. From then on they remained in filtered sea water until May 28, when the feeding experiments were started.

Every morning a carboy (about 19.5 liters) of fresh ocean water was brought to the laboratory and run through the mussel jar at a slow rate, i. e., during the following twenty-four hours. This container, as well as the stock solution, was vigorously aerated, so that the plankton was well suspended. At the end of the twenty-four hours numerous living dinoflagellates could be found at the bottom of the carboy. The mussels opened up shortly after the addition of the water and seemed to be feeding actively throughout the experiment. Copious amounts of well formed fecal matter were present every morning, which showed the usual picture under the microscope, i. e., débris and half digested plankton organisms. The digestive glands, which at the beginning were of a pale color, turned gradually darker, demonstrating the storage of plankton pigments in these glands. The temperature of the water rose during each day from about 12 C. to room temperature, i. e., to about 18 C. (once to 22 C.) without apparent harm to the shell-fish.

The results of the experiment are detailed in table 2. It is clearly evident that the mussels which had turned practically atoxic in the laboratory became poisonous again after ingestion of fresh unfiltered sea water. While the extractives from the digestive gland (measured in milligrams per hundred cubic centimeters of mussels) did not increase noticeably, the poison content rose at least twenty times and probably more. It is unfortunate that at the start of the experiment the mussels contained a substance injurious to mice but not identical with P II.[2c] It is barely possible that the treatment with chlorine gave rise to this poison, although this is the only one of several tests in which such an effect was noted. Even if all the poison demonstrated were considered to be the paralytic shell-fish poison, the increase in toxicity from an average lethal dose of 22 mg. to one of 0.85 mg. after fifteen days of feeding is remarkable.

The quantitative relationship between the number of organisms and the amount of poison is also evident from a comparison of the figures in table 2. Since the genus Gonyaulax was strongly suspected of being the direct cause of the toxicity, the numbers of these organisms only are tabulated. The actual number of Gonyaulax catenella ingested by any of the mussels is evidently an unknown quantity. The amount of poison in one organism, as will be seen later, varies but may be taken to be one three thousandth of a mouse unit. If it is assumed (*a*) that the mussels filter with the same efficiency as the plankton net, (*b*) that

3,000 Gonyaulax catenella yield 1 mouse unit and finally (*c*) that the loss of poison by excretion is negligible, the agreement between the calculated toxicity and the values found experimentally is very satisfactory. During the sixteen days of feeding a total of 213 mouse units of poison accumulated in a standard sample of mussels (two medium-sized mussels), while 193 units could be accounted for by the number of Gonyaulax catenella present. The discrepancies are well within the

TABLE 2.—*Accumulation of Poison in Mussels*

		Volume of Mussels, Cc.		Mouse Units in Plankton		Mouse Units in Mussels#		Toxicity of Mussels, Av. L. D., Mg.	
Date	Gonyaulax Catenella per Liter	Present	Withdrawn	Per 18 Liters of Sea Water	Per 100 cc. of Mussels	"Accumulated" Calculation	Experimental Finding	In Experiments	From San Francisco Beach
May 28	1,475*	450	...	8.8	2.0	2.0	...	....	....
May 29	2,120	450	...	12.7	2.8	4.8	...	....	....
May 30	.....	...	112	....	....	....	0	22 (symptoms atypical)	....
May 31	737	338	...	4.4	1.3	6.1	...	....	....
June 1	761	338	...	4.6	1.4	7.5	...	....	2.5
June 2	...	...	100	....	....	....	7.3	16 (symptoms typical)	....
June 3	782	238	...	4.7	2.0	9.5	...	....	....
June 4	962	238	...	5.8	2.4	11.9	...	....	....
June 5	4,140	238	...	24.8	10	21.9	...	....	0.8
June 6	24,000	238	...	144	61	83	...	....	....
June 7	20,100	238	140	121	51	134	104	2.0 (symptoms typical)	....
June 8	1,390	98	...	8.3	9	143	...	....	....
June 10	2,530	98	...	15.2	16	159	...	....	0.17
June 11	1,855*	98	...	11.1	11	170	...	....	....
June 12	1,180	98	...	7.1	7	177	...	....	....
June 13	1,235*	98	...	7.4	8	185	...	....	0.47†
June 14	1,290	98	...	7.7	8	193	...	....	....
June 15	.....	..	98	....	....	....	213	0.85 (symptoms typical)	....
June 17	.....	..	...	....	....	....	...	....	0.26‡

* The value is interpolated.
\# It may be seen that the values set in bold face type in these two columns approximately check.
† Mussels were taken high up.
‡ Mussels were taken low down.

limits of error of the various determinations and may be explained by the variability in the poison content of the dinoflagellates and in the rate of filtration through the plankton net and the mussels. For the sake of comparison the toxicity of the mussels collected during the time from San Francisco Beach is also given in table 2. The higher toxicity in these shell-fish is evidently explained by the larger numbers of Gonyaulax at their disposal and the higher initial toxicity. The results lend strong support to the theory that Gonyaulax, and in this particular case Gonyaulax catenella, is the direct source of the poison in the mussels.

SUMMARY

Mytilus californianus which lost toxicity in filtered sea water in the laboratory became strongly poisonous after being fed fresh unfiltered ocean water for sixteen days. The increase in toxicity could be quantitatively correlated with the numbers of Gonyaulax catenella in the water.

DEMONSTRATION OF PARALYTIC SHELL-FISH POISON IN PLANKTON

By Dr. Sommer

The early attempts at demonstrating the mussel poison in the sea water were unsuccessful, since at that time the behavior of the poison toward various chemicals was not sufficiently understood. With the finding of the poison in sand-crabs,[4] the adsorption of the toxic principle on sand became apparent. At the same time Mueller [5] investigated the behavior of the poison toward various adsorbents and found permutit particularly useful for the process of purification. Further experiments on adsorption, and particularly on elution (unpublished results by Sommer, Silverberg and Monnier), led to practical procedures by which the demonstration of the poison in the plankton could be attempted with more promise of success.

EXPERIMENTAL OBSERVATIONS

Extraction of Poison from Plankton.—The first experiments were performed during the summer of 1930 on a pier at Halfmoon Bay.

With the help of a hand pump, large amounts of surface water were filtered through a no. 5 and a no. 25 net, suspended in series. The residues from the no. 25 net of as much as 4,000 liters of water were extracted with slightly acidified absolute methyl alcohol. In two separate attempts, at the beginning and toward the end of the poison period of 1930, this method did not yield a trace of poison. Likewise negative was a similar test on July 25, 1931.

In the summer of 1933, at a pier on San Francisco Beach, a second series of toxin tests on plankton was started. Filtration was done through a no. 15 and a no. 25 net. The possibility that the poison became adsorbed on the microscopic sand of the seston [21a] was at this time appreciated, and the method of extraction was modified accordingly. At first, separate extractions were made with acid methanol, intended as a solvent for the poison from the plankton, and with a concentrated solution of potassium chloride, the latter an eluent for the substance from the sand. The bulk of the potassium chloride was removed from the latter extract by the addition of acetone up to 80 per cent, at which concentration the poison is soluble.[1] Later on the material was extracted alternately with acid methanol and small portions of potassium chloride. The extracts were then pooled and set on ice for the crystallization of the bulk of the potassium salts.

21 a. Seston is the total suspended matter in the water, as obtained by filtration through a plankton net (Kolkwitz: Pflanzenphysiologie, ed. 2, Jena, Gustav Fischer, 1922).

By these procedures the paralytic shell-fish poison was definitely demonstrated for the first time in plankton residues in July 1933. This poison in plankton was compared with an extract of poisonous mussels and found to be identical with it in all respects, i. e., solubility, stability and symptoms in mice. The fact that it was found in larger amounts when the toxicity of nearby mussels was greater also speaks for the identity of the poisons from the two sources. A comparison with the number of Gonyaulax catenella (table 3) seems to indicate that the substance can be demonstrated by this method when the count of these organisms is sufficiently high.

Besides the paralytic shell-fish poison, at least one other substance injurious to mice was demonstrated in some of these samples. This

TABLE 3.—*Demonstration of Mussel Poison in Plankton*

Date	Water Filtered, Liters	Net Number	Mouse Units Demonstrated	Type of Symptoms	Total Number of Gonyaulax Catenella	Number of Gonyaulax Catenella per Dose	Toxicity of Mussels from San Francisco Beach (Av. L. D., Mg.)	
July 8, 1933	400	..	55	P I and P IV	1,200,000	22,000	July 7, 1933	1.1
							July 11, 1933	1.1
July 15, 1933	400	..	46	P I	1,300,000	28,000	July 17, 1933	0.5
Feb. 17, 1934	960	25	15	P IV	310,000	21,000	Feb. 17, 1934	3.3
Feb. 17, 1934	960	15	12	P I ?	310,000	26,000	.....	...
April 23, 1934	240	..	0	....	5,000	.....	April 25, 1934	9.5
Oct. 26, 1934	400	..	22	J IV	8,800	400	Oct. 26, 1934	>50
July 19, 1932	Beach sand		0	....				
March 26, 1934	Beach sand		0	....				
Spring, 1934	Navicula*		0	....				
July 4, 1934†	Noctiluca		20	P IV				
July 28, 1934†	Plankton		55	Atypical				

* The sediment in stored crude sea water was used.
† The samples were from Puget Sound.

toxic principle has been designated P IV;[2c] it resembles closely true mussell poison as to the symptoms in mice, except that it causes no heart block. P IV seemed to be present in plankton more often than P I, but by no means consistently, so that an injurious effect of the potassium chloride and other reagents, as well as of the sea water, can be ruled out. Two control experiments on wet sea sand likewise showed no harmful effects. It is not unlikely, therefore, that more than one physiologically active substance is present in extracts from plankton. In experiments 1 and 2 (July 8 and 15) of table 3 the mussel poison predominated so that it could be demonstrated. In experiment 1 two fractions were obtained, one of which produced the typical symptoms of mussel poison.

While the presence of the paralytic shell-fish poison in the seston was herewith fairly well established, the source of the substance was still in doubt. Since 1 mouse unit of poison represents not more than

approximately 1 microgram (γ), i. e., 0.001 mg., of substance, the small amount demonstrated might well have been adsorbed on the sand and was not necessarily contained in the bioseston.

The third series of experiments, performed early in the summer of 1935, finally demonstrated that one fraction of the bioseston is the direct source of the mussel poison. A detailed study of the methods of extraction, which will be published elsewhere, led to a simplified procedure for the demonstration of the poison in the plankton. This demonstration is at present carried out as follows:

The filter residue is centrifugated and the sea water removed as quantitatively as possible. The mixture of sand and plankton is extracted with 50 per cent

TABLE 4.—*Relation of Toxicity of Plankton to Gonyaulax Catenella*

Date, 1935	Water Filtered, Liters	Mouse Units Isolated	Average L. D., Mg.	Number of Gonyaulax per Liter	Percentage of Gonyaulax	Total Number of Gonyaulax	Number of Gonyaulax per Av. L. D.
May 30	150	5.2	7.2	183	10.4	27,450	5,200*
June 5	150	1,041	0.036	14,230	83.5	2,136,000	2,050†
June 6 a.m.	0.38§	4	...	24,000	84.5	9,130	2,280
June 6 p.m.	300	2,024	0.036	28,200	87.6	8,460,000	4,180†
June 7	0.414§	1.9	...	20,100	82.6	8,320	4,380
June 8	0.585§	#	...	1,390	49.0	813	>813
June 10	0.585§	#	...	2,590	52.5	1,498	>1,498
June 12	0.585§	#	...	1,180	45.0	690	>690
June 21	30	1.7	...	156	37.0	4,680	2,820
June 28	30	10	...	680	38.2	20,400	2,040
July 1	30	5	...	575	43	17,250	3,450
July 8	75	9.65	11.3	432	26.5	32,400	3,360
July 15	30	1.4	...	100	5.3	3,000	2,140
July 22	60	0.93	25	35	...	2,100	2,260
July 29	150	0.84	...	<8	...	<1,200	<1,430¶
Aug. 5	150	2.5	...	50	...	7,500	3,000
Aug. 12	150	1.65	...	21	...	3,072	1,860

* This sample stood for eight hours before extraction and may have been partly decomposed.
† Three extracts were made of these samples.
§ This was an aliquot part of a 19 liter sample.
... Leaders indicate that the figures were not determined.
Poison was not demonstrated.
¶ Owing to the small number of Gonyaulax in the water, the chance distribution of organisms on the slide (no Gonyaulax seen) may account for the discrepancy in this result.

methyl alcohol containing 1 per cent of concentrated hydrochloric acid, in a boiling water bath for a few minutes. About 30 cc. of liquid is usually sufficient for a sample, obtained from 30 to 100 liters of sea water. Larger amounts are extracted with two or three successive portions of acid methyl alcohol. After cooling, the insoluble portion is removed by centrifugation and, if necessary, by filtration, and the solution evaporated on the water bath to dryness. The residue may be washed free from some of the lipoids and pigments with chloroform. The dried residue is weighed, taken up in water and tested on mice, as described for mussel extracts.[2c]

The poison thus obtained differed in no way from mussel poison. The symptoms in mice, the solubility in water and alcohol, the insolubility in ether and chloroform, and the instability toward alkali were

confirmed. Since the potency of the extracts from plankton and from mussels is of the same magnitude also, there is no reason to doubt the identity of the two substances.

The experiments performed during the summer of 1935 are compiled in table 4. It is evident that they were undertaken with various purposes in mind and are therefore not strictly comparable. The main result, however, the constancy of the relation between the number of Gonyaulax and the amount of poison as measured in mouse units, is at once apparent. The number of Gonyaulax per lethal mouse dose varied from about 2,000 to 4,000, which is a fair agreement considering the sources of error in counting the organisms, in extracting and in testing the poison.

The first experiment (May 30) proved that even with relatively small numbers of Gonyaulax the poison can definitely be demonstrated by extracting with acid aqueous methyl alcohol. Since the sample had stood for about eight hours in sea water at room temperature, it is believed that some of the poison was destroyed before extraction. The experiments of June 5 and 6 were done in order to obtain a relatively large amount of plankton poison. The test of June 5 may be described more in detail:

Surface water to the amount of 150 liters (i. e., about 20 buckets) was filtered through a no. 25 net and the residue collected in a volume of 72 cc. It was inadvisable to filter more water at one time, since much seston decreased the speed of filtration rapidly by clogging the pores. After rapid transfer to the laboratory the sample was centrifugated, and 1 cc. portions of the clear supernatant fluid were injected into two mice. Both animals died with typical symptoms of mussel poisoning. (Ordinary sea water is not lethal to mice.) Considering the weight of the animals and the time until death, 110 units of toxicity was demonstrated in the 72 cc. The residue was then washed with 8 cc. of distilled water; 12 mouse units of toxicity was present in this wash water. To the sediment was added approximately 20 cc. of 50 per cent methyl alcohol containing hydrochloric acid to the strength of a tenth of normal, and the centrifuge tube was set into a bath of boiling water. The heat was turned off after a few minutes and the bath allowed to cool to room temperature during three hours. After centrifugation, the clear liquid was evaporated in a weighed glass dish on top of a steam bath until just dry. Several successive portions of chloroform were added to the dish and much of the fat and pigments dissolved by rotating and decanting. After a short period on the steam bath, the dry residue was weighed; it amounted to 28 mg. In this first extract 780 mouse units of toxicity was found. The potency of the poison thus equaled 36 micrograms. A second extract of the residue with aqueous acid methyl alcohol yielded 7.4 mg. with 113 units (= 66 micrograms), and a third extract, 2.8 mg., containing 26 lethal doses (= 108 micrograms). A total of 1,040 mouse units was thus obtained from this sample. The count, performed on an aliquot part of the sample before extraction, revealed a total of some 2,100,000 Gonyaulax catenella, so that 2,050 of these organisms yielded 1 lethal dose. The amount of poison received in these extractions was ample to perform the identification tests, inclusive of a feeding experiment on mice. The

potency of the product exceeded that of the most poisonous mussel extract of the season; the toxicity of the latter amounted to 160 micrograms on June 7, while the sample of plankton showed a minimal lethal dose of 36 micrograms. It is evident that a poison of this potency shows much greater promise of being isolated by chemical means than the poison from shell-fish extracts, which always contain much material from the mussel livers. The main contaminants of the plankton poison are sea salts, which are easier to remove than organic extractives.

The experiments of June 6 and June 7 to 12, inclusive, show clearly that as little as 400 cc. of sea water are sufficient for demonstrating the poison if the number of Gonyaulax present is sufficiently large. In these tests, an aliquot part (1.5 or 2 cc.) of the routine daily sample of 19 liters (concentrated to about 70 cc.) was mixed with an equal volume of acidified absolute methyl alcohol and worked up as already described.

The remaining experiments of table 2 need no further comment. The poison was demonstrated whenever a sufficient number of toxiphoric organisms were present. Gonyaulax catenella increased again in numbers toward September 1, and dropped from then on, so that on October 7 no poison could be found in the plankton in as much as 300 liters of sea water. It appears, then, that toxic extracts can be obtained whenever 4,000 organisms of the poisonous Gonyaulax species can be collected. The amount of sea water which can be filtered is limited, of course, by practical considerations.

Amount of Poison Contained in Gonyaulax Catenella.—If it is assumed that no other living or nonliving particle than Gonyaulax contains any poison, the number of suspected organisms to yield 1 mouse unit of poison is approximately 3,000. If it is further assumed that the mouse unit is contained in 1 microgram of pure substance,[22] the percentage poison content of Gonyaulax catenella can be approximated. An average length of 32 microns, a diameter of 42 microns [3] and a specific gravity of 1.1 of Gonyaulax catenella indicate that the mass of one organism equals about 0.033 microgram, i. e., 30 organisms per microgram. Since, on an average, 3,000 Gonyaulax yield 1 mouse unit, it follows that the poison content is of the order of 1 per cent of the weight of the organism.

The most toxic plankton extract obtained killed mice in amounts of 36 micrograms. These extracts were made from plankton which contained about equal numbers of dinoflagellates and diatoms. Eighty-five per cent of the former consisted of Gonyaulax catenella, giving a total percentage of 42.5 of the suspected organisms. Assuming that all plankton organisms yield roughly the same amount of dry extract, a pure culture of Gonyaulax catenella should be expected to yield a poison of 36 times 42.5/100, i. e., 15 micrograms. The poison would there-

22. Sommer and Meyer.[2e] Mueller.[5]

fore amount to 6.5 per cent of the extractives, which seems a reasonable figure, if it is considered that much salt accompanies the poison in the plankton extracts. To summarize:

3,000 Gonyaulax have a wet weight of 100 micrograms.

3,000 Gonyaulax yield 15 micrograms of dry extract, which equals 15 per cent of the wet weight.

3,000 Gonyaulax yield 1 microgram of pure poison, which equals 1 mouse unit.

3,000 Gonyaulax yield 1 per cent of their wet weight, or 6.5 per cent of the weight of the extractives, as pure poison.

These figures are tentative values. How wide the limits are within which the toxicity of the plankton organisms vary remains for further research.

Other Plankton Poisons.—The questions naturally arise (*a*) whether Gonyaulax is the only genus which contains the paralytic shell-fish poison and (*b*) whether any other poisons may occur in plankton. While the first question must be left open at present, for lack of evidence, the second one may, with some probability, be answered in the affirmative. There is definite evidence of at least one more toxic substance in extracts from plankton. It has been mentioned that a second poison (P IV) was found in 1933 and 1934. The same substance could not be demonstrated with the improved extraction method in 1935. The samples of plankton in which the Gonyaulax poison did not interfere with the tests were not large enough to yield a sufficient amount of P IV. Indirect evidence, furthermore, points to the presence of other toxic substances in plankton. Mention was made [2c] of the poison found in mussels and sand-crabs (P III) from La Jolla in May 1933. It is clearly distinct from P I and P IV so far as it acts after an incubation time of from six to thirty hours. Since the shell-fish were collected during a time of "red water," caused mainly by large numbers of Ceratium and Prorocentrum, it is not unlikely that this substance originated from these organisms. Whether P II, the poison found in the mussels most often in the winter time, when P I is absent, has its source in the plankton also, or is a normal constitutent of mussel livers, remains for further investigation.

As far as we are aware, the literature does not reveal any instances of toxic action by dinoflagellates or by marine plankton in general. Fresh water plankton, on the other hand, especially the blue-green algae, has definitely been linked to several intoxications of domestic animals. Fitch and co-workers [23] have summarized the literature on this toxic "waterbloom" and have described recent cases of poisoning from it in

23. Fitch, C. P.; Bishop, L. M.; Boyd, W. L.; Gortner, R. A.; Rogers, C. F., and Tilden, J. E.: Cornell Vet. **24**:30, 1934.

fresh water lakes in Minnesota. In the last case the poison was also demonstrated in the filtered water. The symptoms in animals differed from those caused by the Gonyaulax poison, but they were severe enough to prove that a powerful toxin was present in this case also. Death ensued in guinea-pigs in from seven minutes to several hours.

Connection with Epidemiological Data.—The question naturally arises how far the outbreaks of mussel poisoning recorded in the world literature can be linked with the occurrence of large numbers of Gonyaulax. It is fairly certain that the recent outbreaks in California were due to this organism, although this has been proved definitely only for the cases in 1932 and 1933. Investigations after the outbreak in Oregon in September 1933 could not be started in time to prove any connection with the plankton. While during the last days of September and the first days of October the mussels still contained maximal amounts of poison, the samples of plankton were surprisingly low in dinoflagellates and diatoms; few organisms of the genus Gonyaulax were present. No information on plankton was available during the outbreak in Alaska in 1934. The severe cases of poisoning in Ventura County occurred on May 17, 1936. On May 21 the mussels were of an unusually high degree of toxicity, while the water, which was exceedingly clear, contained some 30 Gonyaulax per liter (mostly Gonyaulax polyedra). The dominant organisms were Prorocentrum micans and Ceratium. It is evident that negative findings in the plankton do not disprove the connection between Gonyaulax and poison in the mussels, since the organisms in plankton may drop to insignificant numbers from one day to the next, while the shell-fish lose their poison only gradually. The maximal number of Gonyaulax always precedes the maximal amount of toxicity in the mussels.

While the similarity of the recent outbreaks on the Pacific Coast speaks for an identical causative organism, such a finding may be questioned for the European outbreaks. The latter [1] were caused by mussels which originated regularly from estuaries and inner harbors. In these cases it must be assumed that an organism is responsible which has its habitat in brackish waters. Whether a species of the genus Gonyaulax was responsible can, at this time, only be surmised. The habitat of this genus is described as neritic, with some species occurring in brackish and in fresh waters. Gonyaulax tamarense has been found by Lebour in the estuary of the River Tamar only. It is not entirely unlikely that an organism of this sort may have been responsible for the European outbreaks. From the identity of the symptoms caused in man and animals by the Pacific and the European poison, it is only logical to look for organisms of the same genus; in the Pacific outbreaks, an organism with a neritic habitat, and in the case of the North Sea, one which prefers brackish waters.

SUMMARY

Methods have been worked out for the extraction and demonstration of the paralytic shell-fish poison from plankton.

Frequent samples of plankton collected during the summer months of 1935 yielded amounts of poison roughly proportional to the numbers of the dinoflagellate Gonyaulax catenella present. On an average, 3,000 of these organs yielded 1 mouse unit of poison.

The facts point to certain species of the genus Gonyaulax as the source of the paralytic shell-fish poison. Evidence for the presence of other toxic substances in the plankton is presented.

PARALYTIC SHELL-FISH POISONING

HERMANN SOMMER, PH.D.
AND
KARL F. MEYER, M.D., PH.D.
SAN FRANCISCO

This investigation of the paralytic form of shell-fish poisoning was begun after an outbreak of mussel poisoning [1] in July 1927 in the neighborhood of San Francisco. Various phases of the study have been carried on to this date, so that at present the records of nine consecutive seasons are available. The problem is of importance from the standpoint of public health, as well as of great biologic interest.

EPIDEMIOLOGY

In order to visualize the significance of shell-fish poisoning as a problem of health on the Pacific Coast, it is well to remember the following facts: During and following the investigation of the outbreak that occurred in 1927, numerous communications from physicians and other persons brought out the fact that mussel poisoning along the northern coast of California is a rather common phenomenon. Besides those recorded previously,[1] numerous single and group intoxications are recalled by the inhabitants. Mass intoxications in Indians and single ones in white settlers which occurred about fifty years ago are also well remembered.

Table 1 summarizes the recent epidemiological data.[2] Of the years under investigation, 1927 took the heaviest toll. One hundred and two persons fell ill; six died. During 1928 no cases were reported. It must be remembered, however, that the quarantine on the sale of mussels was then still in force and that the people showed little desire, after the experience of 1927, to gather shell-fish. In July and August 1929 sixty-two cases of poisoning were reported; one man died after consumption of mussels, while three died from the effects of eating clams. As pointed out in a preliminary report,[3] these are the first unquestionable cases reported in the literature on paralytic shell-fish poisoning

From the George Williams Hooper Foundation, University of California.

1. Meyer, K. F.; Sommer, H., and Schoenholz, P.: J. Prev. Med. **2**:365, 1928.

2. From the statistics of the California State Department of Health, supplemented by reports from private sources.

3. Meyer, K. F.: Am. J. Pub. Health **21**:762, 1931.

TABLE 1.—*Cases of Paralytic Shell-Fish Poisoning on the Pacific Coast 1927-1936*

Miles from San Francisco or Crescent City, Calif.	Area	Locality	1927 July 17	1928	1929 July 7	1929 July 21	1929 Aug. 4	1930 July 27	1931	1932 June 19	1932 July 3	1933 July 9	1933 Sept. 4	1933 Sept. 11	1933 Sept. 17	1933 Sept. 24	1933 Oct. 1	1934 July 8	1934 July 15	1935	1936 May 17	Total	
1,500 N	Alaska	Douglas Is., Juneau	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	4 (1)*	..	..	4 (1)	12 (2)
1,400		Tyree, Admiralty Is.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	8 (1)	....	..	..	8 (1)	
108 N	Oregon-Northern California	Coos Bay, Ore.#	..	..	..	..	..	..	..	..	..	..	..	..	..	..	d.a.‡	....	....	..	..	..	15 (1)
105		Cape Arago, Ore.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	d.a.	....	....	..	..	..	
69		Port Orford, Ore.	..	..	..	..	..	..	..	..	..	..	..	..	..	4 (1)	....	....	....	..	..	4 (1)	
52		Musselrock, Gold Beach, Ore.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	....	....	..	..	3	
38		Pistol River, Ore.	..	..	..	..	..	..	..	..	..	..	..	..	..	1	....	....	....	..	..	1	
21		Brookings, Ore.	..	..	..	..	..	..	..	..	..	..	2	..	..	..	....	....	....	..	..	2	
14		Smith River, Calif.	..	..	..	..	..	..	..	..	..	..	..	3	..	..	....	....	....	..	..	3	
287 00		Crescent City, Calif.	..	..	..	..	..	..	..	..	..	..	..	..	2	..	....	....	....	..	..	2	
135	Central California	Fort Bragg†	..	..	..	..	..	..	..	1	..	..	..	..	..	..	....	....	....	..	..	1	213 (11)
104		Point Arena	..	..	..	..	..	2	..	..	..	..	..	..	..	..	....	....	....	..	..	2	
76		Stewart's Point	..	..	..	..	..	..	..	..	4	..	..	..	..	..	....	....	....	..	..	4	
64		Fort Ross	..	..	..	..	1	..	..	..	..	..	..	..	..	..	....	....	....	..	..	1	
56		Russian River	..	..	..	..	5	..	..	..	..	..	..	..	..	..	....	....	....	..	..	5	
48-51		North of Salmon Creek	4	..	..	9	2	..	..	..	10 (1)	..	..	..	..	..	....	....	....	..	..	25 (1)	
47		Bedega Bay#	..	..	..	..	6 (3)	..	..	..	3	..	..	..	..	..	....	....	....	..	..	9 (3)	
41		Dillon Beach	23	..	..	2	4 (1)	..	..	..	..	..	..	..	..	..	....	....	....	..	..	29 (1)	
41		Tomales Bay†	..	..	..	11	..	..	..	..	1	..	..	..	..	..	....	....	....	..	..	12	
32 N		Point Reyes	8 (1)	..	..	6	..	..	..	..	1	..	..	..	..	..	....	....	....	..	..	15 (1)	
00		San Francisco	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	..	
11 S		Musselrock, Salada	5 (1)	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	5 (1)	
14		Rockaway	12 (1)	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	12 (1)	
16		Pedro Point	14 (2)	..	..	..	..	..	..	5	..	..	..	..	..	..	....	....	....	..	..	19 (2)	
18		Green Canyon	..	..	1	..	..	..	..	1	..	7	..	..	..	..	....	....	....	..	..	9	
20		Montara	12	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	12	
21		Moss Beach	1 (1)	..	..	..	..	..	..	1	..	..	..	..	..	..	....	....	....	..	..	2 (1)	
22		Pillar Point	6	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	6	
30		Lobitos Beach	..	..	..	9	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	9	
32		Tunitas Beach	5	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	5	
40		Pescadero	11	..	1	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	12	
44		Pigeon Point	..	..	5	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	..	5	
90		Monterey Peninsula	1	..	..	..	..	..	..	..	13	..	..	..	..	..	....	....	....	..	..	14	
330	Southern California	Big Sycamore Canyon, Ventura Co.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	....	....	....	..	3 (2)	3 (2)	
		Total	102 (6)		62 (4)			2		40 (1)		22 (1)						12 (2)			3 (2)	243 (16)	

* The number in parentheses is the number of deaths.
\# These were cases of clam poisoning.
‡ Domestic animals only were poisoned.
† The exact locality is unknown.

from clams. During 1930 only two cases of mussel poisoning were reported, and in those cases the effects were mild. In 1931 none came to the attention of the state department of health. In 1932 forty cases, with one death, were recorded; twenty-two cases, with one death, were the toll for the year 1933. In 1934 twelve cases, two of which ended fatally, were reported. For 1936 three cases, with two deaths, are on record. From these statistics of the past nine seasons it seems that yearly occurrences of intoxications from the consumption of shell-fish from certain parts of the Pacific Coast are the rule rather than the exception.

In regard to the origin of the poisonous mussels encountered in recent years, it becomes at once evident from table 1 that four distinct areas have been involved: (*a*) the coast between the Monterey Peninsula and Fort Bragg, to be called herein the central California area, (*b*) the coast between Crescent City, Calif., and Coos Bay, Ore., designated as the Del Norte-Oregon area, (*c*) the Alaska area and (*d*) the southern California area. A consideration of the cases described by Vancouver, reporting from the southern coast of British Columbia in 1793, indicates that toxic mussels may occur in any section of the coast between southern California and Alaska. The discussion will at present be limited to the two central areas involved during the last nine years.

During this time the outbreaks along the central California shore have been of sufficient frequency to allow certain conclusions. From the statistics of 1927 as well as from evidence gathered during the following years, it is clear that the "disturbance" centered in the neighborhood of the Golden Gate and extended roughly 100 miles (160 kilometers) to the north as well as 100 miles to the south. (Too much stress should not be placed on the single case from Fort Bragg, as only scant information as to the exact locality of the mussels involved was obtained.) The center and also the limits of the area have varied from year to year. The northern and southern boundaries are indistinct; the toxicity seems to taper off at both ends. This may in part be due to the fact that the coast-lines toward the borders of the area in the north and in the south for about 70 miles (112 kilometers) are sparsely inhabited and inaccessible. If the intensity were the same over the whole line, the larger centers of population, Santa Cruz and Monterey in the south, Fort Bragg in the north, would be expected to show larger numbers of cases. That the area of poisonous mussels is in reality not much larger is shown by the complete absence, up to 1936, of intoxications in southern California and in Eureka in the north. The latter fact also indicates clearly that the outbreaks in the central California area are separate from the Del Norte-Oregon outbreak. The fifteen cases which occurred in the fall of 1933 were distributed

over a coast-line of approximately 70 miles, or 110 miles (177 kilometers) if the poisonings of domestic animals near Coos Bay are included. The poisonings were like those of 1927, generally severe. The southern California outbreak of May 1936 occurred too recently to permit more than mention of the incidence.

A comparison with the European outbreaks shows again that the California epidemics involved vastly larger areas. Epidemiological records from Leith, Scotland,[4] Wilhelmshaven, Germany,[5] Oslo, Norway,[6] and Calais, France,[7] and experimental findings by two of the authors [8] show that in the European cases a port, estuary or even only a part of such a body of water yielded poisonous mussels at a single outbreak. Furthermore, it is well worth while to call attention briefly to the geographic distribution of the various areas. The localities of the European outbreaks lie exclusively between the latitudes 51 degrees (Calais) and 60 degrees (Oslo), while most of the recent cases on the Pacific Coast are confined within the latitudes 34 and 45 degrees, i. e., considerably farther south. The two localities involved in Alaska lie from 57 to 58 degrees north. The difference in latitude is apparently of slight importance, since the temperatures of the water along the coast of California and Oregon in summer are abnormally low and do not differ much from the summer temperatures of Alaskan waters.

Several interesting observations may be made concerning the relation of time and incidence of outbreaks. It has been pointed out that the poisonings of 1927 seemed to start south of the Golden Gate and within from two to three days appeared at the northern mussel beds. A similar shift was perceptible in the years 1929 and 1932, with the difference that two and even four weeks elapsed between the outbreaks south and north of San Francisco. The Del Norte-Oregon cases of 1933 showed no regularity in this respect. The observation recorded in the previous paper that mussel poisoning seems to occur only in the summer months, between May and October, received no exception from the records of the last nine years.

It will be noted in table 1 that all the cases which occurred during or about a certain week-end have been combined and are given under the date of that particular Sunday, especially if no definite information was available. This is all the more permissible since in the majority of cases shell-fish are collected at week-end outings and consumed during the course of the following week. The number of cases in one

4. Combe, J. S.: Edinburgh M. & S. J. **29**:86, 1828.

5. Wolff, M.: Virchows Arch. f. path. Anat. **104**:180, 1886.

6. Thesen, J.: Arch. f. exper. Path. u. Pharmakol. **47**:311, 1902.

7. Netter, A., and Ribadeau-Dumas, L.: Compt. rend. Soc. de biol. **63**:81 and 195, 1907.

8. Wolff.[5] Thesen.[6]

outbreak in a certain locality is of little significance; it is not so much a measure of the toxicity of the shell-fish as of the number of persons in the party and neighbors who were presented with mussels. With this point in view, one typical case of shell-fish poisoning may be of the same significance as a large number of cases.

Other factors having influence on the number of intoxications are the weather and the tides. Conducive to a large epidemic is a coincidence of fair weather and favorable low tides at the week-ends. This unquestionably led to the large number of cases in 1927, 1929 and 1932, when these conditions were essentially fulfilled. On the other hand, it is likely that unfavorable tides kept the numbers down during the Del Norte-Oregon outbreak, when extremely poisonous mussels were involved.

Observations made on the outbreak in 1927 could generally be substantiated in the following years. Fresh mussels and shell-fish kept for a few days were equally toxic. All the mussels were derived from the open shore of the ocean and were of the species Mytilus californianus. Not a single poisoning occurred from eating mussels of the species Mytilus edulis, gathered in San Francisco Bay or other bays. It is needless to say that many of the victims were experienced in the gathering of shell-fish and that all the poisonous samples of mollusks examined at the laboratory were in excellent condition. The presence of a nauseating pungent odor described in the former paper could be confirmed in only a few instances. In this respect the majority of the mussels did not differ from nontoxic ones. The statement made in 1928 that the liver of the toxic mollusk is always larger than that of the normal mollusk, and usually more friable, could be substantiated in almost every case. This is, however, a characteristic much too inconspicuous to the untrained eye to be of general usefulness as a distinguishing feature.

All clam poisonings were caused by Saxidomus nuttallii (Washington clam) from Bodega Bay. In 1929 there were three separate outbreaks of two cases each, and in 1932, three single cases. The high mortality from this shell-fish in 1929 (i. e., three deaths from six intoxications) may in part be explained by the fact that two of the victims had consumed whole clams in the raw state; both died from the effects. In two other cases (with one death) it was reported that alcohol had been consumed along with the clams, which may with reasonable certainty be expected to intensify the action of the poison.[9]

9. Definite information is lacking in regard to the rôle which the consumption of alcohol may have played in some of the outbreaks. That attempts at treatment with alcohol or with caffeine should be avoided has been pointed out (Prinzmetal, M.; Sommer, H., and Leake, C. D.: J. Pharmacol. & Exper. Therap. **46**:63, 1932).

On the other hand, the high mortality might well have been due to the fact that poisoning by clams appeared as a complete surprise, while the persons affected by mussels were for the most part well aware of the cause of their symptoms.

The duration of the period of toxicity in mussels may not readily be judged from the available statistics on cases in man, since an outbreak in one locality is usually sufficient to warn subsequent visitors to the coast and to prevent further disaster. From a study of the cases in 1929 and 1932, however, it is evident that the poison season may last through two or three successive periods of low tides, i. e., for from two to four weeks. The data so far accumulated indicate that along the Pacific Coast the time during which danger from shell-fish poisoning exists is that from May until October.

It may be recalled here that it is not the first time that toxicity of mussels from one place has been observed over a period of years. Schmidtmann [10] summarized the data available on mussels from the harbor of Wilhelmshaven from 1880 to 1887. Since the first intoxications in man took place in 1885, the information for the subsequent years is undoubtedly more reliable than that for the years previous to 1885. The figures given in table 2, condensed from Schmidtmann's paper, are interesting in the light of the findings in California. Although the methods used did not allow the earlier workers to detect slightly poisonous mussels, the similarity to the observations on the Pacific Coast is striking.

PERIODIC EXAMINATIONS OF SHELL-FISH

Conclusions drawn from the epidemiological records, although of considerable importance from the standpoint of public health, cannot be expected to throw much light on the biologic side of the general problem of mussel poisoning. In order to elucidate the question as to how shell-fish acquire their toxicity, it became necessary to ascertain the exact degrees of toxicity over a prolonged interval of time and to compare the toxicity curve thus obtained with the course taken by the possible causative agents. At the same time the data were expected to supplement the findings of the epidemiologist and to help materially in the prevention of further outbreaks. During the past few years they have enabled the state department of health to issue quarantines ahead of time and to sound warnings against the consumption of mussels. Since a large amount of the poison in shell-fish was desired for chemical work, the approach of poison seasons was detected by frequent tests on animals.

Methods.—The methods for testing shell-fish, improvised during the outbreak of 1927, were gradually improved as experience was gained and as the chemical

10. Schmidtmann, C.: Ztschr. f. Med.-Beamte **1**:19 and 49, 1888.

behavior of the poison became better known. The sensitivity of the test increased from year to year and therefore the numerical values for the toxicity are not strictly comparable over the whole period. The data, nevertheless, give the essential outline of the toxin curve.

The principle of the method used in testing was described in the first paper. It consists in the intraperitoneal injection into mice of measured amounts of the alcohol-soluble extractives of the livers of the shell-fish. During the 1927 outbreak it was learned that the addition of a small amount of acid (hydrochloric) would remove more poison from the mussels; likewise, that evaporation in vacuo was of considerable advantage. The method in use from July 1927 to July 1929, then, consisted in putting a rather large number of whole mussels through the meat grinder, adding ethyl alcohol that had been acidified to the congo acid reaction with hydrochloric acid, and boiling for from one to three hours under reflux. After the liquid had been filtered of the insoluble portion, it was evaporated in vacuo to a pasty brown mass which was weighed out and dissolved in a known amount of water. In order to gain as much poison as possible from any given sample, the alcohol-insoluble residue was often exhausted with further quantities of acid

TABLE 2.—*Toxicity of Mussels in Wilhelmshaven, Germany, from 1880 to 1887*

Year	Peak of Poison Season	Time at Which Mussels Were Found Atoxic	Cases in Man	Deaths
1880	Sept.*	.....	?*	..
1883	Dec.*	.....	?*	..
1885	Sept.-Dec.	..... April 1886	19	4
1886	Aug. (Sept.)	..... Oct. 1886; July 1887	...	..
1887	Sept.	.....	3	1

* The evidence for the data of 1880 and 1883 is not presented; the data are probably based on unpublished clinical evidence or intoxications in domestic animals.

ethanol. During the outbreaks of 1929, this test became too time-consuming and was greatly simplified. The shell-fish livers alone were extracted; methyl alcohol, on account of its lower boiling point, was chosen as the solvent; the acid was omitted for reasons of simplicity, and evaporation was performed on the open water bath. The fats and pigments were removed with chloroform before injection, in order to obtain a more uniform aqueous solution.

Although this procedure did not yield quantitative results, it proved highly satisfactory for testing a large number of samples in a short time. Later on, the problem of demonstrating mere traces of poison arose, calling for a more quantitative extraction, with as little destruction of the substance as possible. Acid methanol was therefore used as the solvent, centrifugation was substituted for filtration, and the number of mussels per sample was greatly reduced.

Sampling of Mussels.—Since it was the primary purpose of these studies to ascertain the possible danger to human beings from mussels, endeavors were made to obtain the most poisonous mollusks from a given bed rather than an average sample. Collections were therefore made at the time of lower low water, usually when the tide was not higher than + 0.5 feet (15 cm.) (heights are reckoned from mean lower low water). Mussels at the lowest possible level were gathered, preferably those which were washed by an occasional wave. Beginning with June 1933, most of the routine samples were gathered from

Lurline Pier [11] in San Francisco, for reasons of simplicity and economy. Standing on the pier and using a wire basket attached to a boat hook with a long pole, the collector can readily pry a few mussels off the piles. Collections can be made at any time except during higher water and rough seas. The level at which these mussels grow is considerably higher than that we have just described for the standard sample, probably more nearly at the +3 foot (91 cm.) mark.

Selection of Standard Sample.—It will be shown later that the variation in toxicity in mussels in a given bed is not large, i. e., not more than ± 50 per cent. It is negligible compared with the variability in different samples of shell-fish, which amounts to as much as 1:3,500 (toxicities ranging from 0.017 to 60 mg.). The standard sample was therefore limited to a few mussels, the extract of which would yield sufficient material for the determination of the toxicity. It became customary to select a number of specimens the combined length of which measured 10 inches (25 cm.).

For the determination of the toxicity of shell-fish, i. e., the minimal amount of extract which will kill a mouse, it is not essential to start with a standardized sample. If, on the other hand, it becomes desirable to know the number of average lethal doses contained in a certain number of a definite weight of mussels, an endeavor has to be made to standardize the sample. The measures which may be considered for this purpose are the length, the weight or the volume of the mussel. Of these, the volume seems to have the fewest objections; it includes the width as well as the length of the shell-fish, and it has the advantage over the weight that the variability due to the shellwater is ruled out. The volume of the mussels is measured by immersion in tap water in a graduated cylinder. The shells of live animals under such conditions are always tightly closed, and no error is likely to occur from inbibition of water. The following procedure is adhered to at present: Mussels are selected the combined length of which measures 10 inches, usually three or four in number. The volume is determined, and the results of the test are expressed in units of poison per hundred cubic centimeters of whole mussels.

Standard Test.—The shell-fish are opened by cutting the posterior adductor muscle, the digestive glands are carefully removed and, without the addition of shellwater or of other tissues, ground up thoroughly with acid methyl alcohol (4 cc. of concentrated hydrochloric acid per liter). Several portions of the liquid are used, so that the residue becomes nearly colorless. The combined residue and liquids, which measure approximately from 60 to 80 cc., are poured into a centrifuge tube. The mixture is brought to a boil by immersion of the tube in hot water and is kept boiling for a few minutes. Next it is gradually cooled to room temperature, preferably in the same water bath. (If necessary the extract may be cooled rapidly and worked up at once, or it may be stored for several days. It should, however, not be kept longer than a week.) After standing for a few hours or over night, most of the alcohol-insoluble substances have settled; they are removed by centrifugation, and the clear dark colored fluid or an aliquot part is poured into a weighed glass dish and evaporated on a boiling water bath. The amount of fluid chosen depends on the time taken for evaporation, i. e., not more than from thirty to forty-five minutes, and the amount of residue expected; 200 mg. is sufficient for a test; from 50 to 200 mg. is usually obtained from a

11. The Olympic Salt Water Company placed its facilities at the disposal of the workers.

mussel. The residue is removed from the water bath before it is entirely dry, i. e., when it is still of a pasty consistency. It is triturated with the help of a glass rod, with several small portions of chloroform, until the latter comes off nearly colorless. The solvent is poured off each time and discarded. This extraction of the lipids and pigments may be readily accomplished if the residue shows the degree of dryness mentioned. Floating particles may, however, be made to settle by the addition of ether. The residue is next heated on the water bath for one or two minutes, with constant stirring, until it is quite viscous. It is hygroscopic and should be weighed as soon as cold. For the injections distilled water ten times the weight of the residue is added, making a solution of approximately 10 per cent.

It is evident that the method can be modified and adapted to specific needs. For the detection of mere traces of poison it may be considered advantageous to evaporate in vacuo; better yet, the poison may be precipitated from the alcoholic solution by the addition of from 5 to 10 volumes of ether. The dried residue may then be directly dissolved for injection. On the other hand, certain errors must be avoided. Grinding of the tissue with sand, as performed in 1927, is of decided disadvantage, since it has been shown [12] that the shell-fish poison is adsorbed by sand in much the same way as by permutit (an artificial sodium aluminum silicate).[13] If large amounts of extract have to be injected, so that it becomes desirable to neutralize the solution, great care must be exercised in doing this, since even a slightly alkaline reaction may destroy 50 per cent of the poison in a short time. In the standard test as done at present the solution is not neutralized for injection. The thorough extraction with chloroform and the subsequent heating should be ample to remove the free hydrochloric acid introduced. It is very likely that the remaining acid is bound to the betaine which is found in large amounts in any extract of shell-fish and which is quite harmless on intraperitoneal injection in the form of its hydrochloride. A fatality from the acid reaction alone has never been encountered.

The average lethal dose of shell-fish poison has been defined [14] as that amount which will kill a 20 Gm. mouse in from ten to twenty minutes with typical neurotoxic symptoms. Besides uneasiness, a wobbling gait, strong spasms and gaspings, the heart block may be used as a differential symptom. It is present only when the dose of poison is not too massive; otherwise the heart is entirely stopped. It develops soon after the heart has been exposed and may last for as long as half an hour. The intestinal peristalsis also is invariably pronounced on autopsy immediately after death from pure mussel poison.

The average lethal dose for the mussel samples tested in the past nine years varied from 0.017 to approximately 60 mg. Whenever larger amounts of extract are injected, the symptoms become more complex and are difficult to interpret. The combined action of several substances may then be responsible for the death of the animal. Of the toxic substances most commonly encountered in extracts of mussels, calcium and magnesium salts are the most prominent. They are likely to occur in any alcoholic extract of marine source. Their average lethal doses for mice on intraperitoneal injection are approximately 10 mg. for magnesium and 20 mg. for calcium salts. Certain of the symptoms, especially in the case of the

12. Sommer, H.: Science **76**:574, 1932.

13. Mueller, H.: J. Pharmacol. & Exper. Therap. **53**:67, 1935.

14. Prinzmetal, M.; Sommer, H., and Leake, C. D.: J. Pharmacol. & Exper. Therap. **46**:63, 1932.

calcium salts, may resemble those caused by sublethal doses of mussel poison. Since Ackermann [15] demonstrated the methylpyridinium base in Mytilus edulis, this substance would be expected to occur in the California variety of mussels also. The lethal dose of the chloride or iodide for mice has been found to range between 5 and 10 mg. The symptoms resemble closely those caused by mussel poison; heart block is also present. A tetramethylammonium salt, isolated by Ackermann, Holtz and Reinwein [16] from actinia, has so far not been demonstrated in mollusks. Its presence in other marine animals might, however, be suspected. It is fairly poisonous to mice, the average lethal dose being approximately 0.5 mg. Muscular paralysis and at best only slight spasms distinguish this substance readily from shell-fish poison.

Since all the substances mentioned normally occur in a standard extract of shell-fish in small quantities only and since the mussel poison is of an extremely high degree of toxicity, the demonstration of the latter, if present, is not difficult. That the principle of the method, namely, the demonstration of this food poison by intraperitoneal injection into mice, is correct has also been demonstrated on numerous occasions. Whenever the mussels reached a high degree of toxicity, i. e., when the average lethal dose was 0.5 mg. of the standard extract or less, the results were checked by feeding the extract to mice. Approximately 40 such doses are needed to kill mice by the enteral route. The fact that cases usually occurred in human beings at such times and never when the average lethal dose was higher, further substantiates the reliability of the testing method. (The occurrence in mussels of unknown poisons, other than the paralytic shell-fish poison, of which one may be a quartenary ammonium salt, has been observed and will be discussed later—page 592.)

Determination of the Average Lethal Dose.—For economic reasons it has not been possible to determine the minimal lethal dose (M.L.D.) of every sample of mussels. For the purpose of this study the average lethal dose (Av.L.D.), a more approximate value, was considered of sufficient accuracy. Experience has shown that this value may be arrived at from the time until death of the mice following injection of a lethal dose or more of the poison. A curve may be constructed, with the values for the time as abscissas and the numbers of doses as ordinates (fig. 1, curve 1). An accuracy of ± 20 per cent can usually be obtained by using three or four mice. Animals given a massive dose, i. e., about 100 Av.L.D., will die in about one minute, while mice which survive thirty minutes will usually recover if left undisturbed. (Such animals have a greater sensitivity to a sublethal amount injected from twelve to twenty-four hours later.) The curve mentioned has been constructed as a mean from numerous standard tests and is essentially correct for acid alcoholic extracts of moderately to strongly toxic mussels. The use of different methods of extraction, neutralization before injection and clam extracts lead to curves with slightly different slopes. Very slightly poisonous mussel extracts often kill after from thirty to fifty minutes; the amount used is then accepted as the average lethal dose. Curiously enough, the injection of an extract from whole shell-fish tissue (without the liver) gives a curve with a very sharp bend; i. e., such extracts kill in a short time or not at all.

The dose-effect curve has been constructed for 20 Gm. mice. Small mice are proportionately more susceptible, while a smaller correction has to be made in the

15. Ackermann, D.: Ztschr. f. Biol. **74**:67, 1922.

16. Ackermann, D.; Holtz, F., and Reinwein, H.: Ztschr. f. Biol. **79**:113, 1923.

opposite sense for larger animals. Curve 2 of figure 1, which is believed to represent these relations with fair accuracy, was obtained from results of injections of constant amounts of poison into mice varying in weight from 10 to 25 Gm. Needless to say, both curves have been smoothed and are intended more as a working tool than as an absolutely correct mathematical expression of the relations.

Field Test.—Another test for shell-fish poison which is of great help in routine examinations consists in extraction of the livers with boiling acidulated water, cooling and injection of 1 cc. of the supernatant liquid. It has been used as a field method when rapid examination of several mussel beds became imperative for the establishment of quarantine measures. For an average-sized mussel liver, 1 cc. of tenth-normal hydrochloric acid plus 9 cc. of water was used. Thorough grinding of the tissue is essential in this test also. One minute of boiling is usually sufficient to coagulate the bulk of the insoluble material; the acid need not be neutralized before injection. Owing probably to the inclusion of poison in the precipitated protein, this test shows about half the number of average lethal

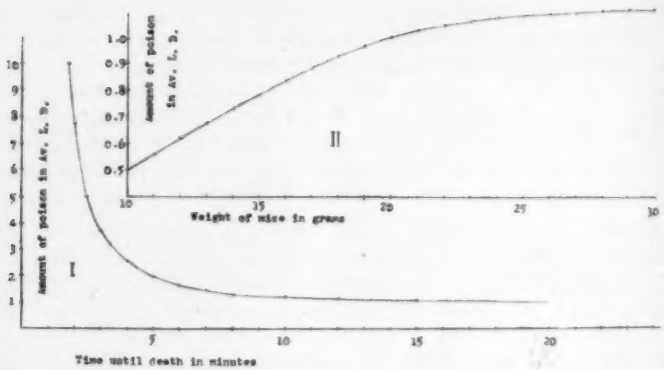

Fig. 1.—*I*, relation of time until death to dose. *II*, relation of weight of mouse to average lethal dose (Av.L.D.).

doses per mussel expected according to the standard test. The ratio is still more unfavorable when neutral extracts are compared.

Comparison of Various Tests.—The methods used for the testing of shell-fish during the past nine years are as follows: (*a*) acid ethanol extract of whole mussels, up to July 1929; (*b*) neutral methanol extract of livers, from July 1929 to September 24, 1930; (*c*) acid methyl alcohol extract of livers, from Sept. 24, 1930, to the present. The ratios of the numbers of doses extracted in the various procedures are by no means constant, but vary with toxicity. Experience has shown that results obtained with procedure *a* have to be multiplied by approximately 10, and those obtained with method *b* by from 3 to 5, in order to make them roughly comparable with procedure *c* as performed at present. These factors have been applied to the figures presented in this paper. Furthermore, many samples of shell-fish had to be shipped to the laboratory and were not in the same state of freshness as those collected in the vicinity. (For the sending of samples 70 cc. bottles containing acid methyl alcohol were dispatched, into which the excised livers of the shell-fish were put.) The amount of shell-water and other

tissues in the sample influences the accuracy of the result somewhat. The personal factor plays a certain part also and is unavoidable in an investigation extending over nine years.

Results.—With due consideration of all these possible variabilities, the toxin curves for the past nine years are presented in figure 2. Needless to say, many more tests have been made than can be presented here; questionable ones and meaningless duplicates have been eliminated. The values for the toxicity represent the reciprocal of the average lethal dose multiplied by 100; i. e., they represent the number of average lethal doses in an average mussel yielding 100 mg. of residue in the standard test. The scale is logarithmic. The danger line, denoting that toxicity at which the mussels can be expected to be dangerous to human beings, has been drawn in arbitrarily, from the epidemiological data. A comparison with table 1 shows the value, 0.5 mg., to be consistent with the human intoxications.

Curve D of figure 2 represents the toxicity during the past nine years in the neighborhood of San Francisco. It is a composite curve of the maximal values found at a given time along a coast-line of about 100 miles south and 100 miles north of the Golden Gate. Curves A, B and C show the toxicity of the mussels, Washington clams and sand-crabs from various places during the years 1931, 1932 and 1933 more in detail. As representative points south of the Golden Gate, Pedro Point and Musselrock, in San Mateo County, and San Francisco Beach have been chosen. The results of tests from the latter two have usually been combined in the same curve. For mussels and clams from the northern area, Salmon Creek and Bodega Bay, in Sonoma County, respectively, have been selected. The results of a few single tests from Santa Cruz, Pascadero and Point Reyes have been added when we deemed it of interest to complete the picture.

The main results of this investigation, then, may be summarized as follows: Every year, between the months of June and September, the mussels along the coast of Central California become poisonous to a degree which is decidedly dangerous when they are consumed by human beings. In most of the years the maximal toxicity is reached about the middle of July in localities south of San Francisco and somewhat later in those north of the Golden Gate. All the curves, near the peaks, are similar in shape. The maximum lasts for one spring tidal period only, i. e., not more than a few days. If the peak extends high above the danger line, the mussels may be poisonous for human beings as long as one month in one place. The rise to the peak seems to occur rather suddenly, although accurate data on this point are difficult to obtain on account of the unfavorable tides. For practical purposes, however, it is sufficient to say that the dangerous toxicity is generally reached during the two weeks elapsing between two consecutive spring

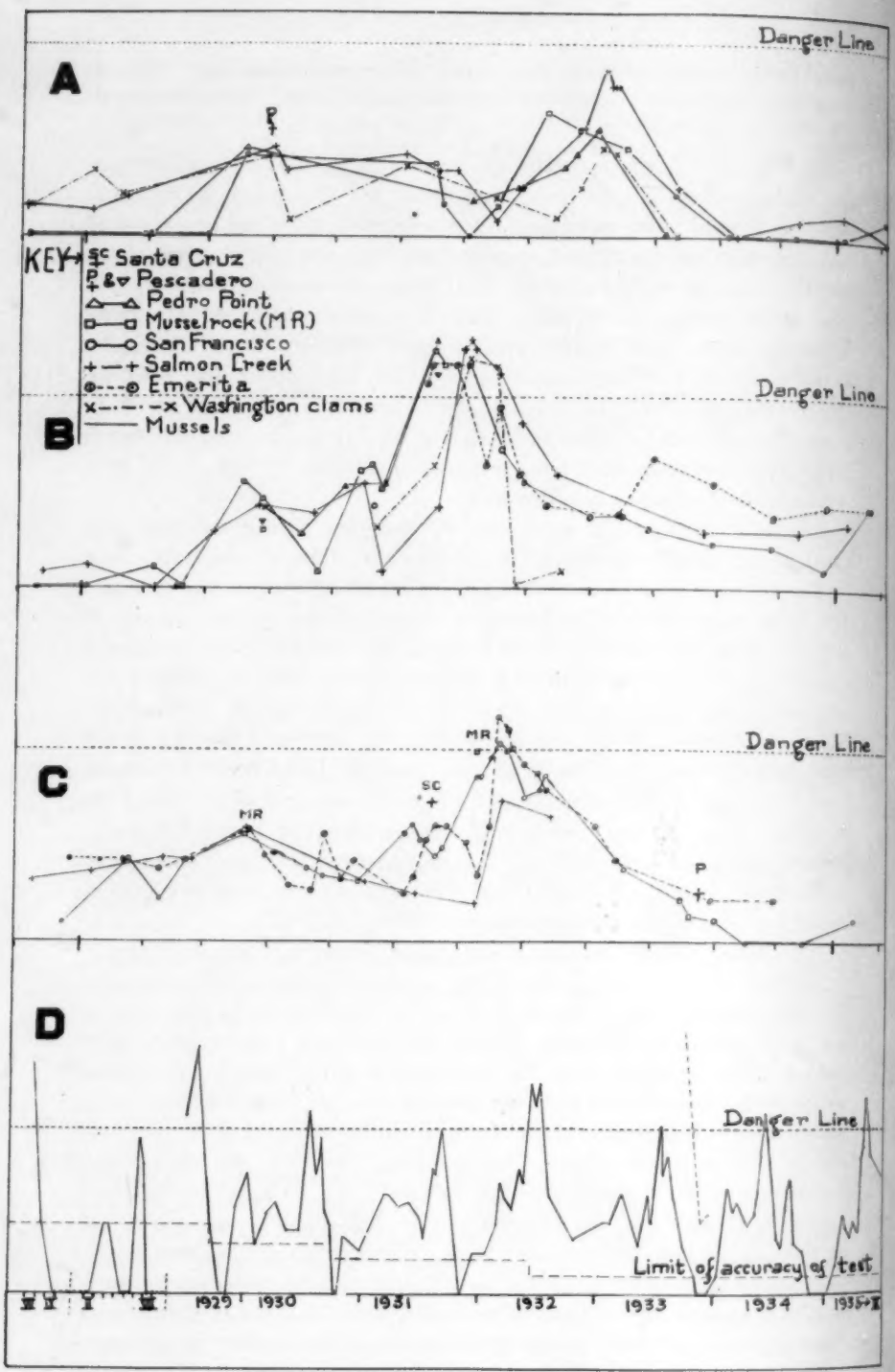

Fig. 2.—Toxicity of shell-fish from various localities (see text on opposite page for explanation of chart).

tidal periods. Detoxification, on the other hand, proceeds at a slower rate, but one month after the peak the toxicity has always dropped below the danger line. It is noteworthy that so far only one dangerous period has occurred each year. From the records of the years 1934 and 1935, in which a second and a third maximum occurred, respectively, it might well be expected that more than one period of danger in one year is possible.

During the remainder of the year the poison curve runs a variable course. Several features, however, are readily discernible. The poison in the mussels is at a minimum or cannot be detected between the months of November and January. Early in the spring the curve rises to a second maximum, well below the danger line, which usually persists until May or June. Then a characteristic drop occurs, generally before the curve rises sharply to the summer maximum.

In an attempt to correlate the toxicity curve with other periodic manifestations, one naturally turns to the meteorological and oceanographic data. From a study of the weather records it is at once apparent that both maxima of toxicity, that in early spring as well as that in summer, fall into a time when the water and air are relatively cool. The temperatures of the water along the coasts of California and Oregon indicate that there are upwellings of colder water to the surface.[17] Consequently the average temperatures of the water along the central coast of California are between 3 and 4 C. lower than the average to be expected from the latitude of the locality. In fact, the average temperatures of the water along the entire region in which mussel poisoning occurs, from central California to Alaska, are remarkably close in summer time, ranging from 10 to 14 C. Most of the measurements made close to the mussel beds in the neighborhood of San Francisco in the early mornings during the poison season fall within this range. It is at least noteworthy that this coast, characterized by strong upwelling currents, should show such a high incidence of mussel poisoning at a time when these upwellings are expected to reach their seasonal maximum.

In regard to the tides, no clear relation can be observed. As a general rule the toxicity may be expected to reach a maximum on or immediately after the second big tide in early summer; but whether there is any causal relationship or whether there is mere coincidence cannot be decided from the data. That the amplitude of the tide and the potency of the poison are not parallel is borne out by the data of the summer of 1927, when the full moon and new moon tides were of nearly equal

17. McEwen, G. F.: Internat. Rev. d. ges. Hydrobiol. u. Hydrograph. **5**:243, 1912. Thorade, H.: Ann. hydrograph. u. marit. meteorol. **37**:17 and 63, 1909.

heights and no "big tides" occurred, and again by the data on the Del Norte-Oregon outbreak, at the time of the autumnal equinox, when tidal differences were at a minimum. It must be concluded, then, that extremely high or low tides are not a *conditio sine qua non* for the toxicity of mussels.

TOXICITY OF OTHER MOLLUSKS

Mytilus edulis, the Bay Mussel.—That Mytilus edulis, which on the Pacific Coast grows only in the quiet water of bays and harbors, has never caused any intoxications was reported in 1928. Since then occasional tests for poison have been made on this species, with uniformly negative results whenever the mollusks were gathered from a bay. The samples, twelve, were collected from a pier at West Berkeley April 13, Nov. 19 and Dec. 19, 1928, May 5, June 3, 10 and 24 and Aug. 10, 1929, July 13, 1931, and June 8, 1932, from Sierra Point, San Francisco Bay, July 22, 1932, and from Bolinas Bay Aug. 7, 1929. Only one sample proved strongly poisonous; it was picked up from a piece of floating lumber which had been swept up on San Francisco Beach, May 30, 1935 (toxicity, 1.2 mg.). How long the wood had been floating in the open ocean is uncertain; it probably originated from piles in San Francisco harbor. The sample was identical in toxicity with Mytilus californianus of the same vicinity, gathered May 29.

Clams.—That bivalves other than mussels may become strongly poisonous was forcefully demonstrated in August 1929, when three men died after the consumption of Washington clams (Saxidomus nuttallii). Subsequent analysis of all the varieties of edible bivalves obtainable revealed that several of them may become dangerously poisonous and that most of them may contain at least traces of the paralytic shell-fish poison. In table 3 the maximal values for the toxicity of clams, gathered mostly in August 1929, are summarized. The figures for 1929 have been corrected to the scale of values obtaining since acid extracts of the livers began to be used. While, therefore, the values can be considered only as approximate, it is clear that the poison in clams may surpass the danger line and reach lethal concentrations, a fact which is well substantiated by epidemiological observations. Tests in which the digestive glands, the white meat and whole clams (of the Washington, horse neck and little neck varieties) were analyzed separately again demonstrated conclusively that the poison is concentrated in the livers. The fact that the large clams (Washington and horse neck clams) are almost always cleaned of the viscera before cooking makes it readily understood why clam poisoning is of such rare occurrence. The epidemiological picture of the week-end of July 4, 1932, at Bodega Bay illustrates this point. On that date, subsequent tests showed, there

were in the possession of the various eating places in this neighborhood large quantities of strongly poisonous Washington clams, which were served to the week-end guests without ill effects. Poisoning occurred in two visitors from the interior valleys who dug and prepared their own clams and probably were not too familiar with the proper preparation of such sea food. The third victim was a local resident who ate

TABLE 3.—*Maximum Toxicity in Clams and Other Mollusks*

Samples	Clam	Locality on Coast of California	Date	Toxicity*
4	Saxidomus nuttallii (Washington clam)	Bodega and Tomales Bays	Aug. 5-7, 1929	0.2 mg.
2	Saxidomus nuttallii (Washington clam)	Bodega Bay	July 5-18, 1932	0.20-0.15 mg.
3	Schizothaerus nuttallii (horse neck clam)	Bodega and Tomales Bays	Aug. 5-7, 1929	1-0.1 mg.
3	Paphia staminea (quahaug; little neck clam)	Bodega and Tomales Bays	Aug. 5-6, 1929	1-0.5 mg.
1	Siliqua patula (razor-clam)	Half Moon Bay	Aug. 8, 1929	1.5 mg.
1	Siliqua patula (razor-clam)	San Francisco Beach	July 7, 1933	2.0 mg.
1	Pholadidea penita (rock-clam)	Half Moon Bay	Aug. 20, 1929	Trace of poison
1	Pholadidea penita (rock-clam)	Half Moon Bay	June 18, 1932	2.3 mg.
1	Tivela stultorum (Pismo clam)	Monterey Bay	Aug. 19, 1929	10 mg.
1	Macoma	Bodega Bay	Sept. 12, 1931	25 mg.
1	Modiolus demissus (horse-mussel)	San Rafael, San Francisco Bay	May 25, 1932	25 mg.
1	Cardium corbis (cockle)	Monterey Bay	Aug. 20, 1929	No poison
21	Mya arenaria (mud-clam)	Bodega, Tomales, San Francisco Bays	From Aug. 7, 1929 to Aug. 12, 1932	No poison
3	Ostrea lurida (native oyster)	Tomales Bay Tomales Bay Humboldt Bay	Aug. 7, 1929 June 6, 1930 Nov. 1931	No poison No poison No poison
1	Haliotis rufescens	Tomales Point and Bodega region	Aug. 4, 1929	No poison
1	Haliotis fulgens (abalone)—livers and muscles	Tomales Point and Bodega region Other localities and dates	Aug. 7, 1929	No poison No poison

* In this and subsequent tables the value for toxicity is the amount of extract that killed a 20 Gm. mouse in from ten to twenty minutes, with typical neurotoxic symptoms.

three raw clams while digging. That two of the persons fatally poisoned in August 1929 consumed whole raw clams has been reported already. (While the liver is usually removed in the preparation of the Washington clam, some of the inhabitants of the coast claim that it is the most "beneficial and tasty part" and is sometimes consumed in the raw state.)

Too little evidence is at hand for a thorough discussion of the possible dangers from the consumption of the popular cockles. One sample of Paphia staminea of August 1929 from Tomales Bay reached the danger

line with a toxicity of 0.5 mg. That this is apparently an extremely rare occurrence is borne out by the absence of any reports of poisoning in human beings from this part of the coast, where these clams are eaten whole and at all times of the year. During the peak of the poison season in 1930 five different samples of Paphia staminea were found to be nontoxic on using the neutral extracts of the livers. Of three samples collected in June and July of 1932, one reached a maximum toxicity of 2.7 mg.

About the cockles (Cardium corbis) and bent-nosed clams (Macoma) too little data are available to draw definite conclusions. From only two tests on Cardium (Monterey Bay, Aug. 20, 1929, and Bodega Bay, Sept. 12, 1931) with negative results it cannot be concluded that this species never becomes poisonous. From its habitat, close to Saxidomus and Paphia, the reverse should be expected. It occurs in too small numbers, however, to be of significance from the standpoint of health. The same observation applies at present to Macoma; no specimens of these species have been received in the laboratory from commercial or private sources. One sample collected Sept. 12, 1931, and consisting of two specimens of Macoma nusata, the bent-nosed clam, and one of Macoma secta, the white sand-clam, showed a toxicity of 25 mg., i. e., about half as much as a nearby sample of Saxidomus. No poison could be detected in one sample of the small Macoma iniquita from Bolinas Bay Aug. 7, 1929. For the time being, then, these clams may be regarded as a possible source of shell-fish poisoning.

Clams of the open seashore (Siliqua patula, Pholadidea penita and Tivela stultorum) should be expected to equal the mussels more nearly in their toxicity. In fact, razor-clams have been found on several occasions to contain only little less poison than mussels from a nearby source, as table 4 shows. While poisoning of human beings by razor-clams has not come to our attention, it has been reported by inhabitants of the coast that domestic animals have occasionally died after eating trimmings of these clams. No further tests were made on Pismo clams, while the sample of rock-clams of June 1932 showed roughly fifteen times less poison than mussels from the same locality. The data, on the whole, seem to indicate that these clams, which live in the surf under conditions similar to those of mussels, do not quite reach the dangerously high toxicity of the latter.

The finding of a small amount of poison in one sample of Modiolus demissus from San Francisco Bay is interesting, all the more since Mya arenaria from similar localities has never in numerous tests yielded a trace of poison. The native oyster, Ostrea lurida, likewise does not seem to become toxic. The inclusion of abalone in this series seemed of interest from the standpoint of health as well as that of biology. The

data, part of which are presented in table 3, indicate that this mollusk does not contain the paralytic shell-fish poison.

Toxin Curve in Saxidomus Nuttallii: Testing the various clams throughout the year was impracticable. Samples of Washington clams from Bodega Bay, however, were received frequently enough to construct a tentative curve of toxicity through 1931 and the poison season of 1932. The results also, represented in figure 2, indicate that the Washington clam follows much the same course in its toxicity as mussels from nearby sources. During that particular period the clams were never free from poison; at times they were even more toxic than the mussels. A comparison of the maximal toxicities during the various years, put together in table 5, is interesting. It is apparent that the higher the toxicity reached by the mussels in any one year the closer is the approach between the toxicity values of the clams and mussels.

TABLE 4.—*Toxicity of Razor-Clams and Mussels from San Francisco Beach*

	May 21, 1932	June 9, 1933	July 7, 1934
Siliqua patula	4 mg.	8.1 mg.	2.8 mg.
Mytilus californianus	4 mg. (Musselrock)	3.6 mg.	1.0 mg.

TABLE 5.—*Maximal Toxicity Reached by Saxidomus and Mytilus*

	1929	1930	1931	1932
Saxidomus nuttallii	0.2 mg.	6 mg.	10 mg.	0.15 mg.
Mytilus californianus	0.05 mg.	1 mg.	1.3 mg.	0.085 mg.

This would mean that clams become dangerous only when the mussels reach exceptionally high toxicity, a fact well borne out by the epidemiological statistics.

Some Other Invertebrates.—At various times earlier in this investigation, several of the common invertebrates living close to the mussel beds, between tide-marks, were investigated for toxicity. They included the common species of barnacles, limpets, snails, chitons, starfish, sea-urchins, worms, sea-anemones from the mussel beds and Medusa and Velella which had been swept up onto the beach. The majority of them were collected at times when the mussels were of slight or medium toxicity. Although the tests were made when the demonstration of the paralytic shell-fish poison in marine material had not yet been satisfactorily perfected, the following conclusions may be drawn: Any demonstration of the typical mussel poison in these animals is doubtful. In the following species a trace of the poison may have been present: Pisaster ochraceus (a starfish), Acmaea cassis pelta (a limpet) and

Mopalia muscosa (a chiton). The toxicity of the starfish should not surprise, since its main food undoubtedly consists of mussels. Wolff [8] in 1886 demonstrated the poison from starfish in toxic mussel beds but not in the vicinity of normal mussels. The analyses of the limpets and chitons need repetition. At present it is sufficient to say that while these animals may contain traces of mussel poison they do not seem to contain as much or more of the toxin than the mussels.

Emerita Analoga, the Sand-Crab.—That the sand-crab may contain the paralytic shell-fish poison has been reported in a preliminary publication.[12] A continuation of the tests for nearly three years has brought several interesting points to light. The toxicity curve of crabs (fig. 2) runs parallel to that of mussels in a general way. In localities where no mussels are available and during unfavorable tides the crabs may therefore serve as an indicator of the toxicity. At times, especially in the winter, Emerita may contain slightly more poison than Mytilus. Sometimes it may be demonstrable in the crabs when it can no longer be detected in mussels; this occurred, for example, Nov. 11, 1933, and Jan. 5, 1935, at San Francisco Beach and Dec. 22, 1933, at Monterey. It should be remembered that the value of the toxicity expressed in milligrams is an arbitrary value and is the result of a test done on livers of mussels. In testing sand-crabs, the digestive organ also is used, usually admixed with a part of the gonads. That the numerical values are nearly equal is only a coincidence. Since one full grown female crab yields from 10 to 30 mg. of extract in the test, the number of average lethal doses in this animal is from a fifth to a tenth of that in the mussel.

While the investigation of poisonous sand-crabs has by no means been exhausted, the tests so far have opened up some new aspects of the problem. It has been mentioned before [12] that sea sand adsorbs the shell-fish poison. In tests of sand-crabs or other material collected on sandy beaches this has to be kept in mind. A sample of sand-crabs of July 29, 1932, for instance, collected in acid methyl alcohol, showed 80 mouse doses of poison in the digestive organs of the crabs, 40 doses in the supernatant alcohol and close to 100 units in the 6 Gm. of sand. As in the case with permutit, this portion of the toxin can be washed out with a strong salt solution; saturated potassium chloride has been used. The rôle of sand along the beach as well as of that suspended in the sea, as carrier of the poison, and the rôle of the sea water as a possible eluting agent need further investigation.

Another point of considerable interest was raised when it was noted that crabs vary greatly as to the amount of pigmentation of the contents of their digestive organs. After the ingestion of food the pigment is apparently of large amount and of a dark color, i. e., of various shades

from brown to almost black, containing a dark brown fluid, while in animals with empty intestinal tracts it is of smaller volume and of a light color or nearly colorless. As table 6 shows, there may be considerable differences in toxicity between the two nutritional stages of the crab. This variability may in part explain the irregular course of the toxicity curve, since it is impossible to obtain a sample of crabs in the same phase of digestion each time. It is not unlikely that this difference is more pronounced at the beginning of the poison season, as the curve in figure 2 indicates. It cannot be explained by rapid excretion of the poison by some crabs since, under laboratory conditions at least, Emerita loses its toxicity only very gradually. The same differences may exist in mussels. They would naturally be hard to detect, since the color of the digestive gland in this animal is no indication of the state of digestion; the gland does not turn light brown except after several weeks of starvation.

Another observation made while testing crabs was the appearance of brown, melanin-like spots on the sides of the body, near the joints

TABLE 6.—*Toxicity of Sand-Crabs from San Francisco Beach*

Date	Intestinal Tracts Full		Intestinal Tracts Empty		Ratio of Numbers of Doses
	Extract of Crab	Toxicity	Extract of Crab	Toxicity	
July 19, 1933	18 mg.	0.20 mg.	19 mg.	2.8 mg.	13:1
July 24, 1933	26 mg.	0.28 mg.	18 mg.	2.8 mg.	15:1

of the legs. These spots have been observed only at such times of the year, and along that part of the coast, at which strongly poisonous crabs (Emerita) occur. Toxin tests made on duplicate samples from the same source, with and without spots, have not given consistent results. More often the animals without spots have been slightly more toxic, so that it appears that this pigmentation is rather a consequence than a cause of the poisoning. Further research is needed in order to decide whether the phenomenon is connected with toxicity at all.

A comparison of the spawning habits of Emerita with those of Mytilus also is illuminating. While a few female crabs with eggs have been observed throughout the year, the optimal spawning season seems to be in midsummer and often coincides with the poison season. Beds of the young of Emerita make their appearance all through the spring and summer months. The mussels, on the other hand, although spawning to some extent at any time of the year,[18] between 1931 and 1934 were found to deposit their sex products mostly in October and Novem-

18. Stohler, R.: Zool. Anz. **90**:263, 1930. Whedon, W. F.: Univ. California Publ., Zool. **41**:35, 1936.

ber, at a time of minimal toxicity. These facts alone seem to disprove the contention that the poison of shell-fish is in any way connected with the reproductive cycle of these animals. Whether the unusually high mortality of all sizes of Emerita which often is observed during the poison season is related to the toxicity or to the spawning cannot be decided from the data on hand and remains for further investigation.

TOXICITY OF SHELL-FISH FROM OTHER LOCALITIES

For the construction of the poison curves in figure 2, mainly three representative areas were selected, namely, Lurline Pier on San Francisco Beach, Musselrock, immediately south of San Francisco, and the coast between Bodega Bay and Russian River, some 50 miles (80 kilometers) north of the Golden Gate. Needless to say, mussel beds of numerous other localities have been tested, but the results are not detailed here since they add nothing essentially new. The results of tests from the following localities, however, are of some interest:

Bolinas Bay.—While the mussels of the open coast and the clams of Tomales Bay and Bodega Bay were still strongly poisonous (Aug. 7, 1929), the samples taken from Bolinas Bay, near the town of Bolinas and two miles north of Stinson Beach, were entirely harmless. Two samples of Saxidomus nuttallii and one each of Cardium corbis, Macoma iniquita and Mytilus edulis were tested.

Point Reyes.—In the tests listed the values for the average lethal dose have been corrected to the present scale:

March 31, 1928	10 mg.
March 9, 1930	3 mg.
Nov. 19, 1930	35 mg.
March 3, 1931	10 mg.

It is evident that at no time of test did the mussels from this source show lower toxicity than those from the Musselrock or the Bodega regions. Oftener they were more poisonous than the mussels from other localities gathered at the same time.

North of the Russian River.—A few samples gathered late in July 1931 seemed to indicate that the "disturbance" was approaching from the north in that year:

Pedro Point, July 26, 1931	25 mg.
Salmon Creek, July 28, 1931	25 mg.
Fort Ross, July 28, 1931	15 mg.
Mendocino, July 29, 1931	16 mg.

A glance at the curve in figure 2, however, shows that the maximal toxicity was reached first at Musselrock and later at Halfmoon Bay and the Bodega region.

Northern California-Oregon Coast.—From this region a series of samples was received in the summer of 1928, which showed moderate and variable toxicity:

Trinidad, Calif.	May 14, 1928	10 mg.
	June 7, 1928	40 mg.
	Aug. 1, 1928	5 mg.
	Aug. 4, 1928	no poison
	Aug. 16, 1928	10 mg. and 20 mg. (2 samples)
Wilson Creek, Calif.	Aug. 17, 1928	20 mg.
	Aug. 2, 1928	10 mg.
	Aug. 8, 1931	25 mg.
Cape Arago, Ore.	June 8, 1928	no poison
	Aug. 10, 1928	no poison

The two tests from Oregon indicate that at least in that year the toxicity did not extend to that northern region. In the fall of 1933, however, the most potent poison so far encountered was found along the coast of southern Oregon (table 7). Few tests were made, and only after the Del Norte-Oregon outbreak. The results substantiate the belief that high toxicity existed in the mussels from Crescent City to Coos Bay, borne out by the severe poisoning in human beings. A comparison of the values for mussel samples from Port Orford and from Cannon Beach shows the great variation in toxicity which may exist between strongly and weakly poisonous bivalves. The former sample was about three thousand times as potent as the latter. A "standard" mussel yielding 100 mg. of liver extract would then contain some 6,000 lethal doses for the mouse; this is evidently an unusually large amount. The values of the average lethal dose for Saxidomus and Schizotherus seem somewhat high, since it was reported that chickens had been killed by clams from the same source. A toxicity of 1 mg. or less should have been expected. Chance in selecting the sample or spoilage in transit may have been responsible for the lower toxicity. Poison tests on sand-crabs all along this part of the coast should have proved of great interest. Unfortunately, no trace of Emerita could be found between Eureka and Coos Bay at the time when the mussels were collected.

San Juan Archipelago.—The finding of practically atoxic mussels at Cannon Beach indicates that the disturbance did not extend farther north than the coast of southern Oregon. The following tests on samples from the coast of Washington, however, disprove this contention:

False Bay, Wash., Aug. 8, 1933	1.2 mg.
San Francisco, Aug. 9, 1933	1.5 mg.
Friday Harbor, Wash., Sept. 17, 1933	7.4 mg.
San Francisco, Sept. 20, 1933	13.0 mg.
Crescent Bay, Wash., June 7, 1935	no poison
False Bay, Wash., July 9, 1935	6.3 mg.
False Bay, Wash., July 28, 1935	7.9 mg.
Friday Harbor, Wash., Aug. 18, 1935	2 mg.

The values for two samples from San Francisco for approximately the same dates in 1933 are quoted for comparison. The agreement between the values for the two localities is remarkable, although evidently only a matter of coincidence. Further studies on shell-fish from this region are highly desirable, all the more, since no epidemiological records are on hand. Likewise no samples have been obtained from British Columbia or Alaska. The presence of the paralytic shell-fish poison in the Alaskan waters is demonstrated beyond any doubt, however, by the case histories of the outbreak on Douglas Island, near Juneau, in July, 1934.[19]

Coast South of San Francisco.—While Musselrock has been the usual place for the collection of mussels south of San Francisco, frequent tests have been made of shell-fish from various other localities (as enumerated in table 1) as far south as Pigeon Point. They have

TABLE 7.—*Toxicity of Shell-Fish of the Del Norte-Oregon Area*

Date	Locality	Shell-Fish	Toxicity	Comment
Sept. 30, 1933.....	Crescent City, Calif.	Mussels	0.055 mg.	
Sept. 30, 1933.....	Smith River, Calif.	Mussels	0.076 mg.	Tide +2.1 feet
Sept. 30, 1933.....	Brookings, Ore.	Mussels	0.071 mg.	Tide +2.1 feet
Oct. 1, 1933.....	Port Orford, Ore.	Mussels	0.017 mg.	
Oct. 1, 1933.....	Cape Arago, Ore.	Mussels	0.040 mg.	
Oct. 1 (?), 1933.....	Port Orford, Ore.	Mussels	0.090 mg.	Whole mussels, sent
Oct. 1 (?), 1933.....	Marshfield, Ore.	Mussels	0.125 mg.	Whole mussels, sent
Oct. 18, 1933.....	Cannon Beach, Ore.	Mussels	50 mg.	
Nov. 15, 1933.....	Crescent City, Calif.	Mussels	10.0 mg.	
Dec. 18, 1933.....	Crescent City, Calif.	Mussels	6.8 mg.	
Oct. 1, 1933.....	Cape Arago, Ore.	Rock-clams	1.7 mg.	Parapholas (?)
Oct. 1, 1933.....	Cape Arago, Ore.	Rock-cockles	1.3 mg.	Paphia (?)
Oct. 2 (?), 1933.....	Marshfield, Ore.	Clams, mixed	2.8 mg.	Saxidomus and Schizothaerus, sent
July 19, 1934.....	Little River Beach, Calif.	Sand-crabs	16 mg.	
July 20, 1934.....	Port Orford, Ore.	Mussels	5.5 mg.	

usually not shown significant variance from those of Musselrock and are not detailed here. Samples from Santa Cruz may at times show a higher toxicity than those from San Francisco, especially at the beginning of the poison season (e.g., in June 1933; fig. 2). They may be useful as an indication of the approach of the disturbance toward the San Francisco area. Sporadic poison tests from the Monterey Peninsula, not reproduced here, only substantiate the epidemiological findings, i. e., that the mussels may be dangerous as far south as this locality.

Southern California.—Shell-fish south of Monterey have not been tested systematically. Scattered samples from Ventura, San Pedro and La Jolla up to May 1936 had not shown any evidence of poison. During May 1933, however, a more extensive investigation was undertaken of the shell-fish near La Jolla. As table 8 shows, the typical paralytic shell-fish poison could not be detected with certainty. Many of these samples contained small amounts of a poison which in its action resembled the paralytic shell-fish poison but, besides, produced other quite characteristic

19. Daily Alaska Empire, Juneau, July 16, 1934.

symptoms. After the mice had received minimal lethal doses they showed the initial symptoms of mussel poisoning, recovering after a few hours. After a lapse of from six to thirty-six hours incoordination and paralysis followed, and death ensued in from eight to ninety hours after injection. Larger doses killed, with symptoms similar to those of mussel poisoning, in from seven to thirty minutes. The cases in which the last-mentioned symptoms occurred have been designated in table 8 as "typical?." It seems, then, that these samples contained a new poison; the question whether traces of the paralytic shell-fish poison were present also must be left undecided.

Quite recently, however, highly poisonous shell-fish have made their appearance in southern California. Preliminary tests on mussels collected immediately after the Big Sycamore Canyon outbreak of May 17, 1936, from Santa Barbara County south to San Diego County, revealed

TABLE 8.—*Toxicity of Shell-Fish from La Jolla, Calif., May 1933*

Date	Shell-Fish	Toxicity	Symptoms
May 17, 1933	Mussel (Mytilus)	60 mg.	Atypical
May 17, 1933	Sand-crab (Emerita)	>60 mg.	None
May 17, 1933	Rock-oyster (Monia ?)	60 mg.	Atypical
May 17, 1933	Porcelain-crab (Lepidopa ?)	>40 mg.	None
May 24, 1933	Mussel	50 mg.	Atypical
May 26, 1933	Sand-crab	42 mg.	Atypical
May 26, 1933	Mussel	40 mg.	Typical ?
May 27, 1933	Horse-mussel (Modiolus)*	30 mg.	Atypical
May 27, 1933	Jack-knife clam (Tagelus)*	40 mg.	Typical ?
May 29, 1933	Mussel	40 mg.	Typical ?
May 30, 1933	Mussel	35 mg.	Typical ?
June 27, 1933	Mussel	40 mg.	Typical ?

* From Mission Bay.

the presence of the paralytic shell-fish poison. Specimens from Big Sycamore Canyon of May 23 showed the highest toxicity ever found, i. e., 16 micrograms per mouse unit, equaled only by the mussels from Port Orford, Ore., of Oct. 1, 1933.

San Francisco Bay.—Shell-fish from San Francisco Bay tested included Mytilus edulis, Mya arenaria and Ostrea lurida. None of these species, either from this or other locations on the Pacific Coast, had ever been found to be poisonous. It was therefore all the more surprising to find a sample of Modiolus demissus from the neighborhood of San Rafael, of May 25, 1932, slightly but distinctly poisonous. This, then, is the only evidence available to show that mussel poison may exist within the bay of San Francisco.

PHYSIOLOGIC ASPECTS OF POISONOUS MUSSELS

Although it has been discussed extensively in the literature since the early reports on mussel poisoning, the question whether the bivalves suffer from a transitory disease is still open. In spite of the lack of

definite evidence, the earlier consensus seemed to answer this question in the affirmative. While in the light of past experience it can scarcely be imagined that mollusks of such intense toxicity for human beings can be normal, the newer knowledge makes this assumption more doubtful. The principal fact which should be kept in mind is that an unusually poisonous mussel of an average length of 3 inches (7.6 cm.) may contain as much as 10 mg. of poison in the digestive gland. Values of from 1 to 2 mg. are probably more nearly the rule. The minimal amounts of poison demonstrable are of the order of 0.002 mg. These calculations assume a minimal lethal dose for the chemically pure poison of 0.001 mg. (Mueller [18] found 0.0012 mg. for the purest amorphous preparation) and represent therefore maximal values. The dry weight of a digestive gland may be taken as roughly 500 mg. At best, then, the digestive diverticula of a highly toxic mussel contains some 2 per cent of its dry weight of a foreign substance to which the animal is apparently entirely immune. It is to be expected, therefore, that any changes observed in the physiologic functions or the histologic picture of the mussel should be slight, if at all demonstrable.

Macroscopic Appearance.—Poisonous mussels cannot be distinguished from normal ones when collected from the mussel beds. They are found with closed valves and firmly attached. (Occasionally, especially in the fall, normal as well as toxic mussels will open their shells soon after being picked and stay open, although able to close the valves if strongly stimulated.) Unusual mortality in the mussel beds has never been observed during the nine years, contrary to the findings with sand-crabs, as mentioned previously. When brought to the laboratory and put into aerated filtered sea water, the poisonous mollusks function normally as far as can be judged from the opening and closing of the shells, the rate of filtration of the water and the formation of feces and pseudofeces.[20] These excreta are usually copious during the first twenty-four hours, since the poisonous state of mussels generally occurs at times when the phytoplankton organisms and the marine bacteria are plentiful. If the poisonous mussels are scrubbed thoroughly and the water is changed frequently during the first few days, their mortality in the laboratory is negligible and not higher than that of normal ones. In fact, mortality is often more pronounced in atoxic mollusks during the fall months, when they are spawning. The rates of the uptake of oxygen and the excretion of carbon dioxide are also the same for toxic and normal bivalves and will be reported in detail separately.[21]

The digestive diverticulum of the poisonous mussels is generally found broader and higher than usual, so that the muscles which traverse

20. Dodgson, R. W.: Report on Mussel Purification, Ministry of Agriculture and Fisheries, London, Fishery Investigation, series 2, 1928, vol. 10, no. 1.

21. Whedon, W. F., and Sommer, H.: To be published.

it seem to cut into the organ. It has a squashy appearance; in fact, on being cut it is found quite fragile. The color does not show any consistency; some very poisonous shell-fish have had remarkably light-colored livers; others of equal toxicity, very dark ones. On various occasions the alcoholic extracts were of a strongly reddish brown coloration. A peculiar odor, although usually present, is no indication of the poisonous quality. It has been variously described as resembling that of cyanide, acetylene, seaweed or plankton concentrate. The odor most consistently found in highly poisonous mussels, especially if these are kept dry for several hours after collection, is the one described [1] as resembling that of an infusion of spoiled meat. Besides the fishy odor of the bivalve, it contains a distinctly acid component, resembling somewhat the smell of fatty acids. While the characteristics described are generally present in toxic mussels, they may ocasionally occur in normal shell-fish and be missing in poisonous samples, so that at present there is no reliable criterion by which the toxicity of the mussels may be recognized.

Microscopic Appearance.—Stohler [22] was the first to show that the stomachs of poisonous mussels are usually found to be full of undigested food material, especially diatoms, while the normal bivalves generally present a picture of more nearly complete digestion. This has been verified in most cases, although the rule is not without exception. Also, the kind of organisms found may vary from those described by Stohler. Definite conclusions can be drawn only after quantitative studies of the available food supply, which is generally more plentiful during the spring and summer months. On the other hand, a detailed investigation of the rate of digestion of the various organisms by the mussels under various conditions is needed before it can definitely be stated whether poisonous bivalves show a larger intake of food or an impaired digestive process.

Microbiologic Aspects.—Normal mussels as well as toxic ones contain a host of protozoa, which apparently live in the mantle cavity as parasites; they are well worthy of a special study. A casual survey reveals an increase in the number of individuals and species during the warmer part of the year and apparently no correlation with the toxicity. The same observation seems to apply to the marine bacteria, which also need further investigation. That pathogenic organisms could not be isolated during the 1927 outbreak has been reported previously.

Physical and Chemical Characteristics.—Greater intake of food and larger digestive glands of poisonous mussels should be expected to show in the weight of the liver extract obtained in the standard test. A mussel

22. Sommer, H.; Whedon, W. F.; Kofoid, C. A., and Stohler, R.: Arch. Path., this issue, p. 537.

sample yields between 150 and 450 mg. of dry extract per hundred cubic centimeters of shell-fish volume. Although during the toxic season the higher figures usually prevail, the amounts of extract plotted throughout the year do not show parallelism with the toxin curve. They run a rather irregular course and depend apparently on some intrinsic factors other than the food supply available to the mussel. The data collected during 1934 and 1935 on the dry weights of the liver extracts will be discussed, together with the results of studies of the food of the bivalves. The chemical and physical characteristics of the various extracts likewise show no perceptible differences paralleling the toxicity. Variations as to color and odor are not consistent. The green color obtained with acids is dependent on the chloroform-soluble fraction. The odor of the poisonous extracts, obtained by heating with alkali, if at all specific for the poison, is always masked by the volatile bases present.

Poison Tests on Single Mussels.—The question naturally arose whether every mussel in a given locality was affected or whether the variation in toxicity was due merely to the variation in the number of a relatively small group of highly poisonous bivalves. It was soon learned, and has been established in numerous instances since, that the poison is distributed fairly evenly in a given mussel bed. This was borne out by:

(*a*) Duplicate tests. Several assays were made of male and female mussels separately. The toxicity was always found the same within the limits of experimental error. In many instances samples were analyzed separately when any distinctive feature made its appearance, e. g., the color or the size of the digestive gland, the amount of sex products in the mantle or the presence of parasites. In no case could a significant difference be found if the mussels in the sample originated from the same locality.

(*b*) Tests of slightly toxic single mussels. These were undertaken, but because of the small amount of extract the number of mice was too limited to give accurate values. In one case the toxicity of the representative sample equaled 30 mg., while that of the single mussels ranged from 15 to 45 mg. (Dillon Beach, April 8, 1931).

(*c*) Daily tests of mussels from one locality. These pointed in the same direction. Table 9 shows the results obtained on a group of mussels from the neighborhood of Bodega Bay. Since not more than three or four mussels were used for one test and the results were computed from the time until death of two or three mice only, the agreement of the results is remarkably close. Besides, the figures show that slight but distinct differences may exist in toxicity in mussel beds 2 or 3 miles (3 to 5 kilometers) apart (Salmon and Scotty Creeks). The sample

from Bodega Rocks, a small island some 600 yards south of Bodega Head, gave a toxicity equal to that found in samples from the mainland; in August 1929 these rocks yielded mussels of an unusually high potency (corrected value, about 0.03 mg.).

Mussels from Different Tide Levels.—This constancy holds only if mussels from approximately the same tide level are compared. That shell-fish collected from widely different levels may show significant variations as to toxicity was pointed out in regard to a sample from Pescadero in July 1927.[1] Specimens gathered from the lowest possible locations, which are swept by the waves most of the time, have on the whole been found more poisonous than those from the upper limits of their habitat, where the water supply may be scarce. On rare occasions only have the highest mussels been more poisonous than the lowest ones. (An example for the latter case is described by Kofoid [23] in a report on mussels from Santa Cruz in 1917.) The usual situation, i. e., more poisonous mussels at lower tide levels, is well illustrated

TABLE 9.—*Daily Tests of Mussels Near Bodega Bay, Calif.*

Date	Locality	Toxicity
Sept. 7, 1931	Salmon Creek	1.1 mg.
Sept. 8, 1931	Salmon Creek	1.0 mg.
Sept. 9, 1931	Salmon Creek	1.0 mg.
Sept. 10, 1931	Scotty Creek	1.5 mg.
Sept. 11, 1931	Scotty Creek	1.5 mg.
Sept. 12, 1931	Bodega Rocks	1.3 mg.

by a comparison between mussels from the San Francisco Beach pier and from Musselrock of the early summer months of 1934. Although this is not the only difference between the two mussel beds, it may be one reason why the pier mussels, at a higher level, have been consistently less poisonous than the ones collected at Musselrock. The ratio of the toxin values is as high as 1:3 (fig. 2).

Excretion of Poison by Mussels.—It had already been demonstrated by the German workers [10] that poisonous mussels gradually lose their toxicity if they are put into sea water in a nontoxic surrounding. During the present investigation detoxification has been repeatedly observed on keeping mussels for a few days, either dry or in sea water. With strongly poisonous shell-fish the excretion of the substance into the water may be demonstrated directly, as the data in table 10 show. The figures probably represent minimal values, since at the slightly alkaline reaction of the sea water it would be expected that part of the poison would be destroyed.

23. Kofoid, C. A.: California State Board of Health, Monthly Bull. **13**:171, 1917.

While the direct determination of the toxin in the sea water can be carried out only in experiments with highly poisonous bivalves, periodic examination of mussels held in the laboratory gives a more definite idea of the rate of disappearance of the poison. Two representative experiments out of some twenty-five are recorded in table 11. Mussels from Musselrock were selected as to uniformity in size and kept in about 1 liter of aerated filtered sea water at room temperature. The water was changed as needed, i. e., once or twice a day at first and every few days later. The standard test performed periodically gave a fair indication of the drop in toxicity as well as of that in the alcohol-

TABLE 10.—*Excretion of Poison by Mussels into Sea Water* *

Date	Mussels	Toxicity	Mouse Units per Cc. of Water After			
			1 Hr.	5 Hr.	24 Hr.	48 Hr.
June 18	17	0.12 mg.	0†	0†	0.9	...
June 23	34	0.20 mg.	..	..	1.2	1.8

* The mussels were from Musselrock, collected in June 1932. The volume of water was approximately 1 liter.
† Poison was not demonstrable.

TABLE 11.—*Detoxification of Mussels in Filtered Sea Water in the Laboratory*

Time, Date	Mussels Tested	Toxicity	Extractives		Mouse Units	
			Per Mussel	Per Sample	Per Mussel	Per Sample
Mussels from Musselrock, Calif., March 26, 1932						
0	5	8 mg.	72 mg.	360 mg.	9.0	45
6	4	7 mg.	38 mg.	150 mg.	5.4	21
14	4	18 mg.	46 mg.	185 mg.	2.6	10
21	4	26 mg.	43 mg.	172 mg.	1.7	6.6
Mussels from Musselrock, Calif., July 9, 1933						
0	3	0.55 mg.	173 mg.	520 mg.	314	946
2	2	0.68 mg.	183 mg.	553 mg.	269	814
8	2	0.87 mg.	220 mg.	440 mg.	253	506
17	2	2.90 mg.	203 mg.	407 mg.	70	140

soluble extractive substances. In general, a drop to one half of the toxicity in ten days may be regarded as the normal rate of detoxification. Occasionally an experiment seemed to show a slight increase in toxicity, but such an irregularity can equally well be attributed to sampling, i. e., to individual differences in the poison contents of single mussels. In none of over twenty tests could any definite increase in toxicity in the laboratory be demonstrated.

Keeping of Atoxic Mussels.—Although the erroneous conception of mussel poison as a postmortem product was dispelled years ago, at the start of this investigation it seemed desirable to submit nontoxic mussels to various conditions of temperature and oxygen tension and analyze them for possible formation of poison. The shell-fish were kept at room

temperature, in the incubator at 37 C. or in the icebox. They were either placed in sea water, with or without aeration, or kept in a dry state. Some samples were exposed to strong sunlight, and others were submitted to sudden changes from cold water to hot air. The experiments were usually continued until the weakness of the reactions of the mussels indicated their imminent death. The results of all these tests can be summarized by stating that in no case was mussel poison formed in the mollusks. Although the tests were performed in 1928 with methods inadequate to detect small amounts of the poison, it is evident from the results and from experience gained generally during these studies that mussels once removed from their natural habitat and brought to the laboratory never show increase in toxicity, regardless of the environmental conditions to which they are subjected. In particular, it is felt that pronounced bacterial spoilage causes an increase in the rate of destruction of the poison, if the latter is present.

Increase in Toxicity of Mussels.—Schmidtmann [10] was the first to show that the increase in toxicity of the mussels in nature may be rapid. By transplanting normal bivalves into the "inner harbor" of Wilhelmshaven, where the poisonous shell-fish occurred at the time, he was able to demonstrate the same potency in both lots after three or four days. Even after twenty-four hours a small amount of poison was present in the transplanted specimens. No attempts were made in these studies to determine the exact rate of increase in toxicity in nature, but from figure 2 it appears that mussels of Salmon Creek in the summer of 1932 showed a hundred-fold increase in about twelve days, while the toxicity of sand-crabs rose from an average lethal dose of 15 to 0.6 mg. in four days between July 11 and July 15, 1933. Although the original toxicity in Schmidtmann's mussels could not be determined with the crude method then available, a rough calculation shows that the two sets of observations are in close agreement. They point out the fact, which is important from the standpoint of public health as well as from that of biology, that slightly poisonous shell-fish may turn into highly toxic and dangerous ones in the course of a few days.

Storage of Added Poisons by Mussels.—That mussels can take up poisons from the surrounding water was first demonstrated by Thesen [6] with curare, strychnine and mussel poison. Although these substances were conclusively demonstrated in the mussels, the experiments mean little from a quantitative standpoint. They were also cut short on account of putrefaction in the aquaria. In one experiment in this laboratory ten mussels in 2 liters of water took up roughly 2 mg. from 60 mg. of strychnine; the remaining quantity of the alkaloid could not be accounted for. Attempts to feed mussel poison have thus far been unsuccessful: the shell-fish close their valves immediately on the addi-

tion of the mussel liver extract and do not open them until the water is changed. These experiments with feeding will be resumed if a more highly purified poison becomes available in sufficient amounts. For the time being it must be considered a possibility that mussels may take up the poison as a foreign substance from the water and store it to a certain degree.

ORIGIN OF THE POISON

From the evidence so far presented it is likely that the poison itself or the factor which causes the toxic condition is transmitted to the mussels through the medium of the sea water, in particular the water of the open ocean. The findings which point in this direction may be summed up briefly:

(*a*) Mussels from lower tide levels are often more poisonous than those from the highest levels. This should be expected, especially during the approach of the poison season. It can well be imagined that a toxicity of the same degree throughout the mussel bed may then gradually be established. Since it was also shown that the bivalves excrete the poison into the water, it may occur that at the end of the poison season the lower mussels may get rid of their toxic substance at a faster rate and become less poisonous than those higher up on the rocks.

(*b*) The findings in clams point in the same direction. Bilvalves which have their habitat in the most seaward locations of bays, i. e., Saxidomus and Schizotherus, contain the highest amounts of poison, while in Ostrea and Mya, at the greatest distances from the open ocean, no traces of poison are ever found. A complete correlation between habitat and toxicity can hardly be expected. Other factors undoubtedly have to explain the lack of quantitative relationships. The differences in toxicity between the coast mussel and sand-crab, on one hand, and the Pismo clam, various razor-clams and rock-clams, on the other, must be attributed to some intrinsic difference in the various species of shell-fish, since the habitats of both groups are very close to each other. In this connection it is very interesting to note that samples of the various kinds of clams just mentioned usually have shown amounts of poison identical with that of mussels at times of moderate toxicity. During the season of high toxicity in mussels and sand-crabs, however, the amounts of poison in these various kinds of clams do not seem to reach more than a tenth of that in mussels. Any explanation of the phenomenon of shell-fish poisoning, then, has to explain the fact that only Mytilus californianus, Emerita analoga, Saxidomus nuttallii and possibly Schizothaerus nuttallii seem to reach high degrees of toxicity.

(*c*) It has been mentioned already that sand-crabs with filled intestinal tracts may be as much as ten times as poisonous as those without stomach contents. Since these animals also strain their food from the

sea water, the foregoing fact may be taken as additional evidence that the poison is carried to the animals by the sea water.

(*d*) Another fact which points to the water of the open ocean as the source of the poison is the absence of the poison in the Mytilus edulis of San Francisco Bay, whenever tested, and of Bolinas Bay in August 1929. That Mytilus edulis growing in estuaries and harbors may become highly toxic is well known from the numerous European outbreaks. In fact, the few investigations made, particularly by Wolff [5] in the neighborhood of Wilhelmshaven and by Thesen [6] near Oslo, indicate that in those cases a drop in toxicity occurred as the open ocean was approached. As a tentative explanation for the reverse condition in California, it must be assumed that the source of the disturbance exists along the shore of the open ocean and is not able to penetrate the Golden Gate to any marked degree. A check of this hypothesis by analysis of a continuous series of samples of shell-fish between the open ocean and the interior of the bay was not attempted for practical reasons. The small but definite amount of poison found in one lot of Modiolus demissus from San Rafael Bay demonstrates, however, the possibility of the occurrence of the poison within San Francisco Bay. A thorough investigation of all parts of the bay in this connection should prove interesting, although from the standpoint of public health such an undertaking is apparently not urgently needed at the present time.

Mussel Poison in Sea Water.—It is natural that suspicion should have been directed against sea water as the possible carrier of the poison. As early as 1886 Wolff [5] tried to demonstrate the toxin by injecting a concentrate of sea water into animals, with negative results. In the light of recent findings his failure is not surprising. The slight alkalinity of sea water alone would have been sufficient to destroy most of the poison on evaporation at a moderately elevated temperature. Furthermore, it has been shown that the paralytic shell-fish poison is strongly adsorbed by sand [12] and permutit.[13] It is therefore unlikely that any poison can be demonstrated in sea water which is in equilibrium with the base-exchanging silicates which may be found in sand, silt, etc. An attempt to demonstrate the poison must therefore be limited to samples of seston,[24] i. e., the residue collected in the plankton net, consisting of micro-organisms (plankton) and microscopic sand and débris (abioseston).

Demonstration of Poison in Plankton.—This will be dealt with in a separate paper. The results of the first successful experiments

24. Kolkwitz, R.: Pflanzenphysiologie, ed. 2, Jena, Gustav Fischer, 1922, p. 222.

25. Footnote deleted.

should, however, be mentioned here, since they seem to add weight to the assumption that the poison is taken up by the shell-fish from the water. After unsuccessful preliminary experiments, the toxin was demonstrated in the seston on several occasions during the poison period in the summer of 1933. The small amounts found, i. e., approximately 1 mouse unit per bucket (8 liters) of sea water, unfortunately do not allow any definite conclusions as to the origin of the poison. It is possible that the plankton, i. e., the phytoplankton inclusive of the adhering bacteria,[26] may have been the original source of the poison; on the other hand, the sand may be the principal carrier of the toxic substance. In this case nothing definite can be said about its origin. It may have been excreted by nearby mussels, clams and sand-crabs; it may have been liberated from dead and decaying organisms, or it may have an entirely different and so far unsuspected origin. It is certain, however, that any poison liberated into the water will largely be bound by the ever present silicates having base-exchanging properties.

POISONS ENCOUNTERED IN MARINE MATERIAL

That in all the cases paralytic shell-fish poisoning is due to one specific poison is reasonably certain from the similarity of the symptoms and circumstances in all the outbreaks of American as well as of European origin. It is another question, however, whether there is more than one poison in the shell-fish extracts when these are tested on animals. In mice after the injection of extracts various toxic effects have been observed which cannot be attributed to the paralytic shell-fish poison. These poisons are listed in table 12. The paralytic shell-fish poison, P I, needs no further discussion here. P II occurs in mussels and, like all the other poisons, can be demonstrated only when P I is absent and does not interfere with the test. Consequently, it has been found in the late fall and winter months. Its potency and/or its amount cannot be large. The smallest amount of standard extract to kill a mouse so far has been 12 mg. This is small enough, however, to exclude the possibility of interference with calcium and magnesium salts. It is not unreasonable to assume that the toxic action is caused by a quartenary ammonium salt. Tetramethylammonium chloride has been found in actinia by Ackermann, Holtz and Reinwein,[16] and similar substances in minute amounts may be of a more general occurrence in marine material. It goes without saying that a mixture of P I and P II may at times be present in mussels in such amounts that symptoms of poisoning with both substances may be observed in mice. The minimal lethal dose for tetramethylammonium bromide has been found to be about 0.5 mg.

26. Waksman, S. A.: Biol. Bull. **75**:127, 1933.

P III is well characterized and stands apart from the other poisonous principles in regard to occurrence as well as symptoms. It is the only poison in this group which has no immediate effect on mice but begins to act after an incubation time of several hours. In the course of these studies it has been encountered only in material from La Jolla, in May 1933. It is barely possible that the "red water" observed during that time [27] coincided with the rise of this toxic substance. The count of dinoflagellates alone rose to almost 200,000 per liter of sea water, and the mussels and sand-crabs were gorged with Prorocentrum micans. This poison also is not very potent or is present in minute amounts only. The minimal lethal dose in no case amounted to less than 30 mg. of crude extract. With regard to the period of incubation, this poison resembles somewhat a toxic principle demonstrated by one of us in

TABLE 12.—*List of Various Poisonous Substances from Marine Sources*

Designation	Symptoms in Mice	Time of First Symptoms	Time Until Death	Occurrence
P I Paralytic shell-fish poison	Central paralysis, strong spasms, heart block	Immediately to 20 minutes	1 to 30 minutes; rarely more	Mussels, clams, sand-crabs
P II.....	Motor paralysis resembling that from tetramethylammonium salt; feeble heart beat	Several minutes to hours	5 minutes to several hours	Mussels
P III.....	Trembling, incoordination, tetanic condition	6 to 30 hours	8 to 90 hours	Mussels and sand-crabs at La Jolla, Calif., May 1933
P IV.....	Resembling P I, but without heart block; feeble heart beat	Same as P I	Same as P I	Plankton [22]

fish eggs—probably from a species of Cottoid. The incubation period of the latter was longer, however, and the poison caused macroscopic changes in the liver (unpublished results, H. S.).

The existence of P IV is not as well established as that of the three previous substances. It was found in various samples of plankton [22] and in amounts not sufficient for a thorough investigation. In mice the symptoms closely resemble those caused by the paralytic shell-fish poison; the heart also survives but does not show the typical block. In this connection it must be emphasized that this symptom is always observed after death caused by 1 or 2 M. L. D. of mussel poison. After massive doses the heart stops at the same time as the respiration. At times, when death ensues after twenty or more minutes, the heart may be found to beat normally for several minutes after the pleural cavity has been opened; the block then gradually develops. These phenomena have never been observed after the injection of P IV. It is barely possible that the large amounts of potassium, calcium and magnesium salts

27. Allen, W. E.: Science **78**:12, 1935.

which are contained in the plankton extracts influence the action of P I so that the heart block is prevented. Further research on plankton extracts is necessary before the existence of the principle P IV can be established definitely.

Stability to acids, to alkali or to heat cannot serve to differentiate the four principles. All are obtained by extracting the material with acid methyl alcohol. During evaporation on the steam bath the reaction is apt to become rather strongly acid and the temperature nearly 100 C. On boiling with dilute sodium hydroxide and subsequent neutralization these principles are inactivated, with the possible exception of P III, which has not been tested in this respect. Extraction of the acid residue with chloroform does not remove any of the poisons.

PREVENTION AND TREATMENT OF MUSCLE POISONING

It is evident from the data presented that the prevention of mussel poisoning is difficult. Without frequent animal tests it is impossible to give warnings of the approach of the poison period. A closed season (in the case of the northern Pacific Coast, from May 15 to October 15) seems more practical. That the old adage which warns against the consumption of shell-fish during the months without R has some foundation and should be extended further is readily understood from the experimental data. Much harm could be prevented also by educating people in the consumption of shell-fish, especially concerning the fact that the livers of mussels and the broth cannot be consumed without danger during the summer months. Although accurate data are lacking for obvious reasons, it is likely that the consumption of only the light meat, even of strongly poisonous mussels, would not lead to serious poisoning. The boiling of mussels with sodium bicarbonate as proposed by Salkowski [28] in 1885 and recently again by Mueller [29] is not expected to find much favor with the coast population, since it largely destroys the delicate flavor of this sea food. Moreover, with highly poisonous shell-fish this procedure is of doubtful value.

In regard to the therapy of mussel poisoning, no new points of view have come to light. Besides aiming at rapid elimination of the poison, the treatment is necessarily symptomatic. Since primarily the respiratory center is paralyzed, artificial respiration should be tried in cases in which the effects are severe. The nervous symptoms and the recovery in three such cases in 1933 have been described by Stegeman.[30] Kelloway [31] has recently described the action of the poison on isolated nerve preparations.

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28. Salkowski, E.: Virchows Arch. f. path. Anat. **102**:578, 1885.
29. Mueller, H.: California & West. Med. **37**:263 and 327, 1932.
30. Stegeman, W.: California & West. Med. **41**:26, 1934.
31. Kelloway, C. H.: Australian J. Exper. Biol. **13**:79, 1935.

COMMENT

In view of the many divergent opinions on mussel poisoning, on one hand, and the few facts known, on the other, it is imperative that more definite information on the various phases of the problem be gathered. This has been the main object of these studies; any conclusions drawn from the experimental material presented are tentative only, and the final word must be deferred until the picture is more complete.

One of the main obstacles to an approach to the problem, i. e., the difficulty of detecting the poison, has been largely overcome. In mussels it is possible to demonstrate as little as from 2 to 4 micrograms per mussel, i. e., about a thousandth of the amount present in dangerously poisonous shell-fish. The finding of the toxic substance in sand-crabs extends the investigation to regions where mussels cannot be secured, along many parts of the coast that are bordered by sandy beaches of great extent. In fact, it seems that Emerita is an even better indicator of the presence of small amounts of mussel poison than mussels themselves. Any attempt at an explanation of the toxicity of mussels must evidently fit the case of the sand-crabs also.

The technic which has been worked out for the demonstration of the shell-fish poison in plankton must be regarded as tentative. It is not unlikely that a more detailed study of the distribution of the poison between exchange silicates and various salt solutions of different concentrations and p_H values will lead to a simplification of the method. The demonstration of the poison in the plankton at times when mussels are moderately toxic is destined to be of great help in tracing the origin of the poison. It will enable the investigator to free himself from the limitation of collection along the shore and allow him to search for shell-fish poison in the open sea, at various depths, inclusive of the sea bottom. Such an undertaking would naturally necessitate the use of a boat and complete equipment for oceanographic studies. It should, however, allow one to answer the question whether the poison emanates from the mussel beds or whether it approaches them from offshore. Along the northern part of the Pacific Coast special attention would have to be paid to the bottom samples of water and mud on account of the strong upwelling current during the summer.

The question whether the sand or the phytoplankton is the carrier of the poison could also be solved, perhaps by taking samples farther offshore. The adsorption of mussel poison on various silicates undoubtedly deserves further study. The affinity of the toxin for permutit and sea sand is remarkable if it is considered that these silicates remove 90 per cent of the poisonous substance from a crude extract of mussel livers, containing large amounts of the chlorides of sodium, calcium,

magnesium and betaine. The interference of microscopic sand in the analysis for certain poisons and substances of basic nature is not generally appreciated. In studies of Haff disease,[32] waterbloom poisoning [33] and similar intoxications, in which samples of mud and water are analyzed, this fact may become of importance. It has been pointed out before that grinding biologic material with sand for analysis should not be resorted to indiscriminately.

Concerning the ultimate source of the paralytic shell-fish poison, little direct evidence is available. If the shell-fish along the central California coast during the past nine years were considered alone, the poison might well be thought of as a normal physiologic constituent of the bivalves, varying in amount greatly but regularly with the season of the year. Since mussels from other localities, notably La Jolla, have never shown a trace of poison, however, this view is untenable. It must be assumed, then, that the toxic substance is of unusual occurrence in the shell-fish, and that the mussel, the clam and the sand-crab belong to the class of accidentally poisonous animals. Whether the poison is formed by them or taken up from an outside source and stored can at present not be decided. From all the evidence herein presented, it is, however, likely that the toxigenic factor, if not the poison itself, is contained in and transported by the water. That this unknown factor is most likely of biologic origin is borne out by the more or less regular increase in toxicity in summer and by the geographic distribution, which exhibits preference for the waters of the temperate zone.

The evidence of the rapid uptake and slow excretion of the poison by mussels explains to a large extent the sudden and mysterious appearance of mussel poisoning. It also explains the fact that no other phenomena accompany its occurrence consistently. The causative factor needs to be present in the water for only a few days to render the mussels dangerously toxic. On the other hand, because of the slow excretion of the substance, the shell-fish may still be poisonous long after the toxigenic substance has disappeared. Even if any outward signs accompany the increase in poison, e. g., discoloration and luminescence in the water, mortality of sand-crabs and unusual odors, they may be too transitory to be noticed, especially by the casual visitor to the coast. It goes without saying that all these phenomena may occur also at other times, when the mussels are harmless.

Deductions from analogy with other poisons are of little avail in the elucidation of the problem, since the paralytic shell-fish poison seems to belong to a category all its own from the toxicologic as well as possibly from the chemical point of view. To summarize briefly: In

32. For review see Flury: Klin. Wchnschr. **12**:1161, 1933.

33. Fitch, C. P.; Bishop, L. M.; Boyd, W. L.; Gortner, R. A.; Rogers, C. F., and Tilden, J. E.: Cornell Vet. **24**:30, 1934.

its powerful action of the respiratory center it resembles some of the most potent alkaloids, but it far surpasses them all in toxicity. The absence of pronounced effects on the heart distinguish it from such strongly poisonous substances as the cardiac glucosides. From the venoms and similar animal poisons it can be differentiated by its lack of action on the blood corpuscles and its apparently low molecular weight. This feature and the stability with respect to heat also readily distinguish the shell-fish poison from the toxins of plants and bacteria, which alone surpass it in potency. There are only two poisons which in some respects resemble mussel poison, and their chemical structure is so far unknown. The poison of the Japanese pufferfish (Tetrodon) causes symptoms similar to those of mussel poisoning in man and animals. Crude extracts of ovaries exert effects (unpublished results) indicating that they contain a highly potent substance, and the most poisonous products obtained by Tahara [34] represent only the first steps in the purification of this poison. The difficulties encountered in the isolation of the two poisons seem to be very similar. Furthermore, the recent report on waterbloom poisoning [33] suggests that strongly poisonous substances may be elaborated by profuse growth of fresh water plankton or by its subsequent decay. Farm animals have died within an hour after drinking from lakes where the plankton grows, and it has been possible to demonstrate the poison in solution in the water. On the other hand, the lethal effects produced by red water [35] on bottom-feeding fish and crustaceans cannot be attributed to a toxic substance of the type of mussel poison, since it has very little effect on cold-blooded animals (Printzmetal, Sommer and Leake; [14] also, unpublished results). Nor is there any close analogy between mussel poisoning and Ciguatera, the poisoning occasionally caused by consumption of certain fish from subtropical American waters, since the clinical symptoms are distinctly different. The same may be said for Haff disease.

While most of the intoxications of aquatic origin mentioned are of rare and sporadic occurrence and can therefore be studied with difficulty only, the problem of shell-fish poisoning along the coast of northern California seems to make a yearly appearance, and it offers excellent opportunity for the study of several phases of marine toxicology.

SUMMARY

We have tabulated and discussed 243 cases of paralytic shell-fish poisoning, with sixteen deaths, that occurred between Ventura County, Calif., and Juneau, Alaska, from 1927 to 1936. Of these, 234 cases were caused by the coast mussel. Nine were caused by the Washington clam.

34. Tahara, Y.: Biochem. Ztschr. **30**:269, 1911.

35. Kofoid, C. A.: Univ. California Publ., Zool. **8**:187, 1911.

Methods for detecting and quantitatively estimating the poison in shell-fish have been worked out. Mussels from numerous places between central California and southern Oregon have been analyzed and toxin curves constructed for the past nine years. The curves for mussels, sand-crabs and Washington clams from localities near San Francisco since 1931 have been specially detailed. Besides the shell-fish mentioned, seven of the common varieties of edible clams may contain the poison in smaller amounts.

Poisonous mussels may in no way be distinguished from normal ones except by the animal test. Mussels subjected to various conditions in the laboratory have never shown an increase in toxicity; they usually show detoxification, the rate of which has been determined. Mussels may take up poisons from sea water.

Strong evidence has been presented which points to the water of the open ocean as the carrier of the poison. Owing to the strong adsorption of the substance on base-exchanging silicates of the sand, it is not likely to occur free in the water. The poison has been demonstrated, at least during the poison season, in the residue from filtration of sea water. Whether it is contained in the plankton or adsorbed in the microscopic sand cannot at present be decided.

Four different principles toxic for mice have been demonstrated in acid alcoholic extracts of shell-fish and plankton.

Measures for the prevention of mussel poisoning have been discussed.

DEVELOPMENT OF LOCAL CELLULAR REACTION TO TUBERCULIN IN SENSITIZED CALVES

WILLIAM H. FELDMAN, D.V.M.
ROCHESTER, MINN.
AND
C. P. FITCH, D.V.M.
ST. PAUL

In a previous report [1] the histologic reactions which occur when tuberculin is injected intracutaneously into spontaneously sensitized cattle were described. The material studied represented specimens of the caudal folds, removed for biopsy seventy-two hours after tuberculin had been introduced. Although the material examined was sufficient to enable us to obtain a fairly comprehensive understanding of the cellular reaction at what was probably the height of the reactive process, it did not provide an opportunity to observe the earlier or later phases of the phenomenon. For the purpose of ascertaining the character of the initial and the subsequent morphologic changes which ensue when tuberculin is injected into the dermis of the tuberculous bovine the following experimental study was made.

METHODS

A group of eleven apparently healthy calves, approximately 3 months of age, was obtained, and each animal was given an intracutaneous injection of tuberculin. None of the animals reacted positively. An adequate ration [2] was supplied, and after the animals had been on the premises for two weeks nine of them were submitted to subcutaneous injection of 2 cc. of a saline suspension of a virulent strain of bovine tubercle bacilli.[3] For control purposes two of the animals were not given the injection of tubercle bacilli. The latter were placed in a separate pen.

Fifty-eight days after the infective bacteria had been introduced, both caudal folds of each of the eleven animals were given an intracutaneous injection of the usual diagnostic dose of mammalian tuberculin, supplied by the Bureau of Animal Industry of the United States Department of Agriculture. Specimens for biopsy were taken from the areas receiving the injection, at the third, sixth, twelfth,

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From the Division of Experimental Medicine, the Mayo Foundation, and the Division of Veterinary Medicine, University of Minnesota, University Farm.

1. Feldman, W. H., and Fitch, C. P.: Arch. Path. **22**:495, 1936.

2. The ration consisted of alfalfa hay, ground grain, skimmed milk, potassium iodide and cod liver oil.

3. The strain had been isolated one year previously from an infected lymph node of a tuberculous cow. The density of the bacterial suspension was comparable to that of tube 1 of the MacFarland nephelometer.

eighteenth, twenty-fourth, thirtieth, thirty-sixth, forty-second, forty-eighth, fifty-fourth, sixtieth and seventy-second hours and on the fifth, seventh, tenth, nineteenth, twenty-first and twenty-eighth days after the injection.[4] Since tuberculin had been injected into both caudal folds of the respective calves it was possible to obtain two specimens for biopsy from each animal. The schedule followed in making the biopsies was designed to provide in most instances an interval of at least thirty-three hours between the surgical procedures on a given calf (table). The tissue removed consisted of that portion of the caudal fold which gave evidence of the most intense reaction. In every instance the incisions for biopsy were made sufficiently deep to obtain a block of tissue extending through the subcutaneous portion. The larger portion of each specimen was preserved in 10 per cent neutral solution of formaldehyde, and a smaller portion of each was placed in Helly's fluid.[4a] After fixation, the respective tissues obtained for biopsy were cut into several portions so as to obtain sections from different parts. The blocks of tissue were dehydrated in dioxane and embedded in rubber paraffin as recommended by Baird.[5] By this method the excessive hardening which frequently makes it difficult to obtain satisfactory sections of skin was avoided. The sections obtained from the tissues which had been fixed in solution of formaldehyde were

Intervals Between Surgical Procedures Carried Out to Obtain Specimens for Biopsy

	Sensitized Calves									Nonsensitized Calves	
Specimen	1	2	3	4	5	6	7	8	9	10	11
First.....	12 hours*	5 days	30 hours	3 hours	10 days	6 hours	18 hours	24 hours	7 days	3 hours	6 hours
Second.....	48 hours*	28 days	72 hours	36 hours	21 days	42 hours	54 hours	60 hours	14 days	72 hours	72 hours

* The time stated in each instance is the time after injection of tuberculin.

stained in the usual manner with hematoxylin-eosin, while those fixed in Helly's fluid were stained by the method of Dominici.[4b] The latter method is of particular value in differentiating the various leukocytes in fixed tissue preparations.

OBSERVATIONS AT NECROPSY

After the last biopsy, all of the animals were killed and submitted to postmortem examination. Marked and in some instances extensive lesions of tuberculosis were present in each of the nine animals which had been given the injection of tubercle bacilli. The portion of the subcutis where the organisms had been introduced gave evidence of caseous

4. Before the specimens for biopsy were taken caudal block anesthesia was induced by injecting from 8 to 10 cc. of a 2 per cent solution of procaine hydrochloride.

4a. McClung, C. E., and Allen, Ezra: Fixation and Fixations, in McClung, C. E.: Handbook of Microscopical Technique, New York, Paul B. Hoeber, Inc., 1937, p. 558.

4b. Slider, E. M., and Downey, H.: Methods for the Study of Leukocytes, in McClung, C. E.: Handbook of Microscopical Technique, New York, Paul B. Hoeber, Inc., 1937, p. 340.

5. Baird, T. T.: Stain Technol. **2**:13, 1936.

or caseocalcareous granulomatous lesions, with extension of the morbid process to the regional lymph nodes; the subscapular and axillary nodes were most often affected. Slight or early lesions of tuberculosis were demonstrated in the lungs of only two of the animals, although the bronchial and mediastinal lymph nodes of four were involved. In three instances the disease was present in the spleen, but in only one instance was the liver affected. Lesions of tuberculosis were not found in the noninfected, or control, calves.

HISTOLOGY

Since the areas of local reaction were obtained at intervals of from three hours to twenty-eight days after the injection of tuberculin, the histologic characteristics of the reactive process are best described in chronologic sequence.

After Three Hours.—There appeared in the tissues immediately adjacent to the vessels in both the papillary and the reticular zone of the dermis a small amount of edematous fluid and a considerable number of polymorphonuclear leukocytes, eosinophilic granulocytes and a few mononuclear cells (fig. 1).

After Six Hours.—Congestion of the smaller arteries was observed, and small areas of hemorrhage were apparent in the reticular zone. Marked perivascular and perineural infiltration of polymorphonuclear leukocytes had occurred around some of the arteries of the reticular zone, and what appeared to be an early endovascular change was noted (fig. 2). Many of the endothelial cells of the intima had assumed a position at right angles to the lumen of the vessel, and the intima had a somewhat thickened, serrated appearance. Polymorphonuclear leukocytes were present throughout most of the upper zone of the dermis, and evidence of early necrobiosis was apparent where excessive accumulations of polymorphonuclear leukocytes were found. A few histiocytes or mononuclear cells were present. An excess of polymorphonuclear leukocytes also occurred in the lumens of the sweat glands.

After Twelve Hours.—The cellular reaction had increased in degree but was still predominantly polymorphonuclear. Edema was present throughout the entire dermis and had extended into the subcutaneous muscle, causing wide separation of the muscle fibers. Between the muscle fibers were large numbers of polymorphonuclear leukocytes (fig. 3*A*). Some extensive perivascular cellular reactions were noted; these were largely histiocytic, although the remnants of atrophic polymorphonuclear leukocytes were present. Histiocytes and lymphocytes were observed in the diffuse edematous areas, and thrombosis of some of the smaller veins was noted (fig. 3*B*). In the upper, or papillary, zone of the dermis a marked, more or less diffuse polymorphonuclear infiltration had occurred, with early necrobiosis evident.

After Eighteen Hours.—The perivascular cellular foci appeared more dense because of the excessive concentration of the cellular constituents. The secondary or smaller vascular channels in the perivascular areas were no longer patent. Polymorphonuclear leukocytes still predominated, although histiocytes and eosinophilic granulocytes appeared throughout the edematous fluid which was present in the midreticular zone. Thrombosis of veins was discernible, and arteries were noted in which there were proliferative endothelial changes. Examination of one artery revealed striking endarteritis with the intima greatly thickened and the medial surface arranged in longitudinal folds (fig. 4).

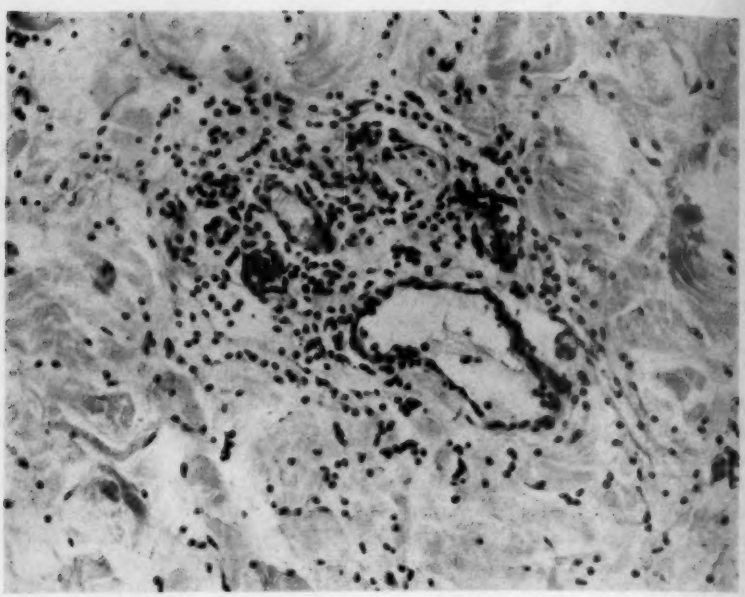

Fig. 1.—Perivascular and perineural reaction in the dermis of a sensitized calf three hours after injection of tuberculin; × 170. The reaction consists of a small amount of edema, many polymorphonuclear leukocytes and a few mononuclears or histiocytes.

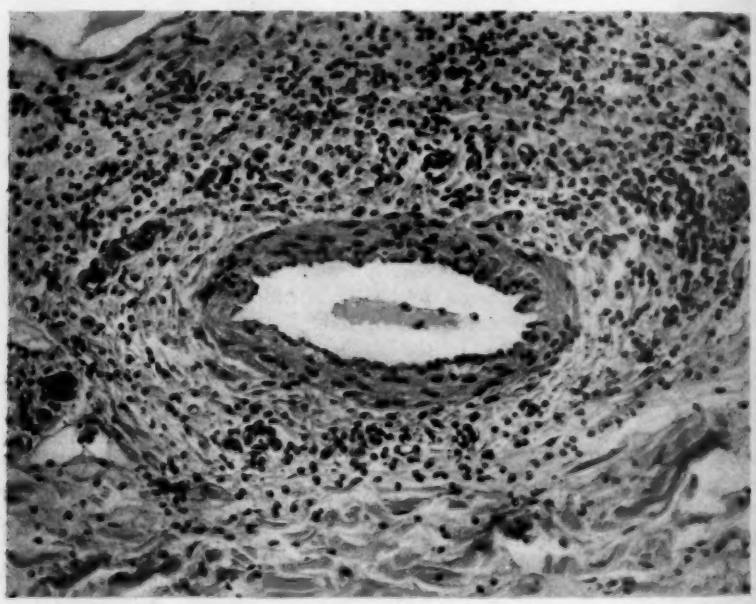

Fig. 2.—Perivascular infiltration of leukocytes in the dermis of a tuberculous calf six hours after injection of tuberculin; × 150. The leukocytes are predominantly polymorphonuclear.

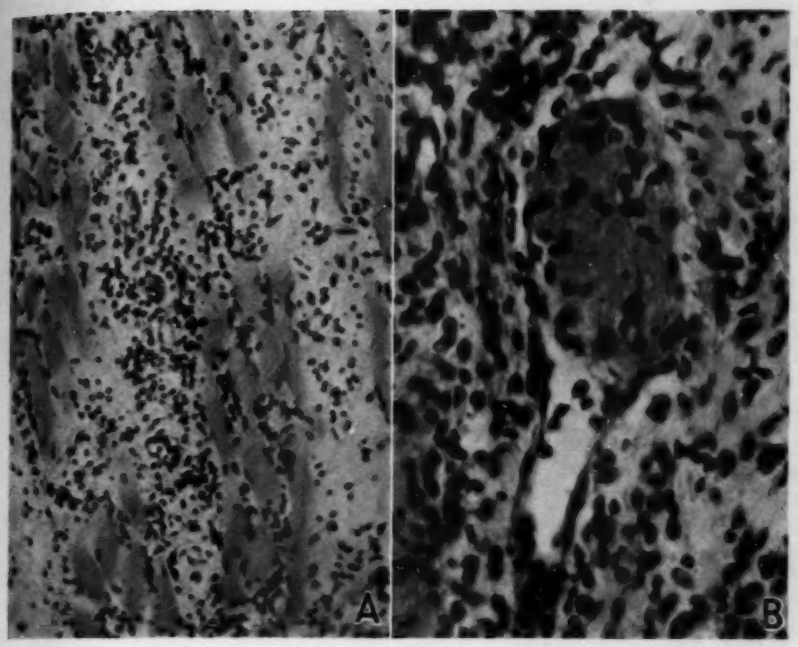

Fig. 3.—*A*, excessive edematous exudation between bundles of muscles in the lower portions of the reticular layer of the dermis; × 150. The cells are largely polymorphonuclears and histiocytes. Tuberculin had been injected twelve hours previously. *B*, partially occluding thrombus in an area of perivascular reaction twelve hours after injection of tuberculin in a sensitized calf. The surrounding cellular reaction consists largely of polymorphonuclears and histiocytes.

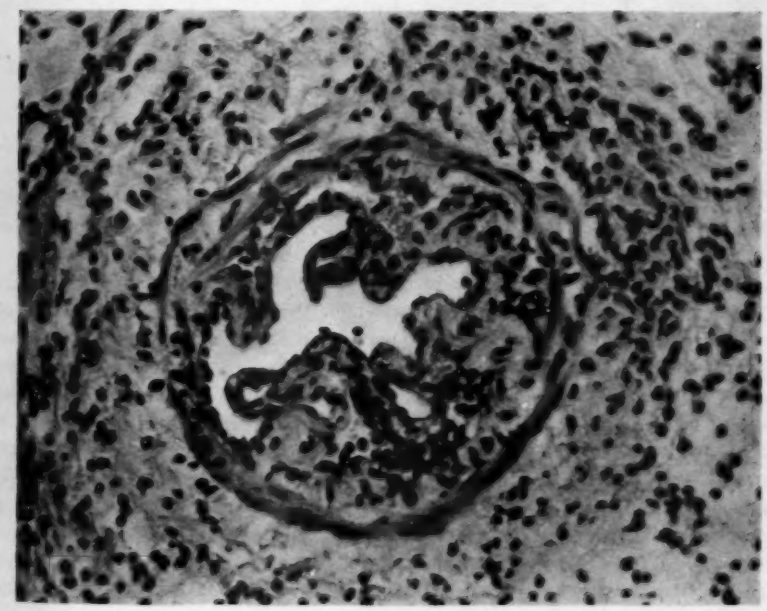

Fig. 4.—Endarteritis in the dermis of a tuberculous calf eighteen hours after injection of tuberculin; × 275. There is marked hypertrophy of the intima.

After Twenty-Four Hours.—With the exception of an increase in the number of histiocytes, the cellular reaction at the twenty-fourth hour was essentially the same as that observed in the tissue removed at the eighteenth hour. Several thrombi were present in which were noted many polymorphonuclear leukocytes.

After Thirty Hours.—A marked increase in the number of histiocytes, some of which gave evidence of mitotic division, and a decrease of polymorphonuclear leukocytes characterized the cellular reaction. Although polymorphonuclear leukocytes remained in the sweat glands of the upper zone of the dermis, the majority of the cells of the reactive process were histiocytic. The polymorphonuclear leukocytes remaining in the perivascular foci had a pinched or atrophic appearance. The eosinophilic granulocytes, many of which were mononuclear, also had increased in number.

After Thirty-Six Hours.—No striking change was noted in the cellular reaction between the thirtieth and thirty-sixth hours. The perivascular deposition of the reactive foci was perhaps more pronounced, but the relative proportion between the histiocytes and the polymorphonuclear leukocytes had not changed appreciably. There was a predominance of polymorphonuclear leukocytes in the edematous fluid of the reticular zone.

After Forty-Two and Forty-Eight Hours.—The reaction had extended to the deeper portions of the reticular layer, and the number of polymorphonuclear leukocytes in the edematous fluid, which was still abundant, seemed reduced. There also occurred in the edematous fluid a considerable number of blood monocytes and a few mononuclear eosinophilic granulocytes. In the muscle fibers which occasionally occur in the lower portion of the reticular layer of the dermis there was a marked cellular reaction. The fibers were separated by edema, and the cells present were polymorphonuclear leukocytes and histiocytes in about equal numbers. Fibrin was noted in some of the larger venous thrombi, and arteries were observed in which the endothelial cells projected into the lumens of the vessels. Small arteries were noted in which the intima was in folds or rugae similar to those observed in the reaction at the eighteenth hour.

After Fifty-Four Hours.—The perivascular foci were largely histiocytic, although a few polymorphonuclear leukocytes, lymphocytes and plasma cells were present. Mitosis was observed among the histiocytes, and many of these cells were becoming epithelioid in appearance. Edema persisted throughout the derma. The polymorphonuclear leukocytes had practically disappeared from the edematous fluid, and mononuclear forms were abundant. The areas of hemorrhage had clotted, and the venous blood contained an excess of polymorphonuclear leukocytes. Thrombosis of the veins was commonly observed.

After Sixty Hours.—The intense perivascular cellular reaction had caused obliteration of most of the smaller vessels, and there was marked thrombosis of the larger veins. The edema was most marked in the lower half of the reticular layer of the dermis, and a marked cellular reaction was present in the intermediate zone between the papillary and reticular layers, resulting in compression of the sebaceous glands and the hair follicles (fig. 5 *A*). The cells constituting the major reaction were largely histiocytic, with a few polymorphonuclear leukocytes still present (fig. 5 *B*). Mitosis was occurring among the histiocytes. Eosinophilic granulocytes, lymphocytes and mononuclear cells also were noted. Although a few polymorphonuclear leukocytes were evident in the edematous areas, the majority of the cells were mononuclears. Endarteritis of the vessels in the deeper portion of the dermis was observed, and in certain areas where the perivascular

reaction was intense many of the arteries were compressed so that the lumens appeared as mere slits.

After Seventy-Two Hours.—The appearance of the reaction had not changed essentially from that observed in the reaction studied sixty hours after the injection of tuberculin. The reactive process was perhaps somewhat more intense, particularly in the papillary zone of the dermis, and although the perivascular arrangement of the foci was still apparent, the cellular accumulations were inclined to be more

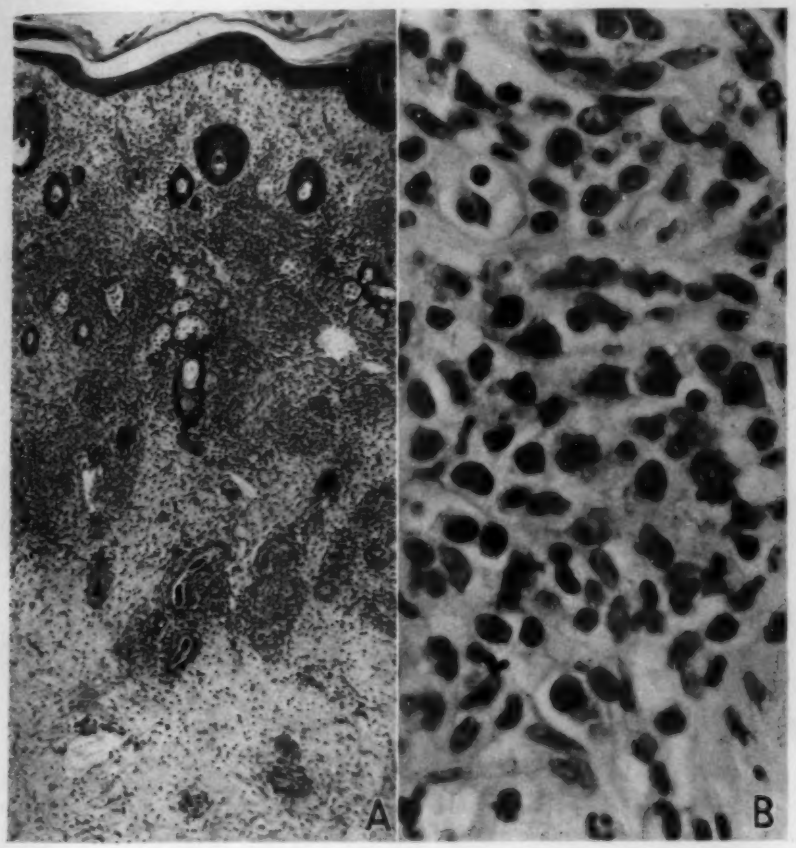

Fig. 5.—*A*, marked cellular reaction in the upper portion of the dermis sixty hours after injection of tuberculin into a sensitized calf; ×40. *B*, higher magnification, showing the histiocytic character of the majority of the reacting cells; ×660.

diffuse (fig. 6 *A*). There was a decrease of polymorphonuclear leukocytes, but they were present in moderate numbers. In many of the reactive foci the histiocytes were more epithelioid than before, and many appeared with clear cytoplasm. The thrombi had contracted and had attracted large numbers of leukocytes, particularly lymphocytes and mononuclear forms and eosinophilic granulocytes. The edema persisted, and threads of fibrin were beginning to form.

After Five, Seven and Ten Days.—The reactive process was still markedly apparent, and there were no essential differences in the character of the cellular foci observed in the material obtained for biopsy on these days. The reaction was characterized by the same perivascular and perineural accumulations of histiocytic cells that were so prominent in the reactions observed at the sixtieth and seventy-second hours. There was, however, a decrease in the amount of edema, and more lymphocytes were present. It was of interest to note, also, that some progression of the process was evident in the cellular reaction of the tissue removed on the fifth day. There was a moderate number of histiocytes with hyperchromatic nuclei, and mitotic division of the cells occasionally could be seen. An increased

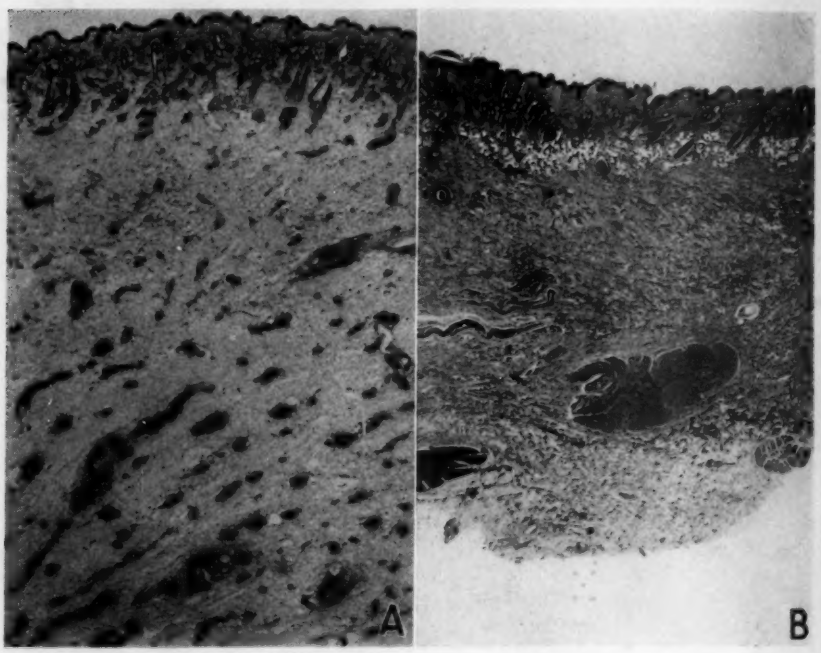

Fig. 6.—*A*, the extent and character of the reactive process seventy-two hours after intracutaneous injection of tuberculin into a tuberculous calf; × 8. *B*, the dermis of a nontuberculous calf seventy-two hours after tuberculin had been injected; × 8. No morphologic changes can be seen.

number of eosinophilic granulocytes were present among the histiocytes in the tissues representing the reactions on the seventh and the tenth days. Cellular invasions of the thrombi were especially prominent in some of the reactive areas. In some of the involved veins the clot had nearly disappeared, and the lumens were gorged with histiocytes. Giant cells of the Langhans type occasionally were seen (fig. 7). These were situated among the cells of the perivascular reaction. A well developed system of reticulum fibrils could be demonstrated among the cells of the inflammatory foci (fig. 8).

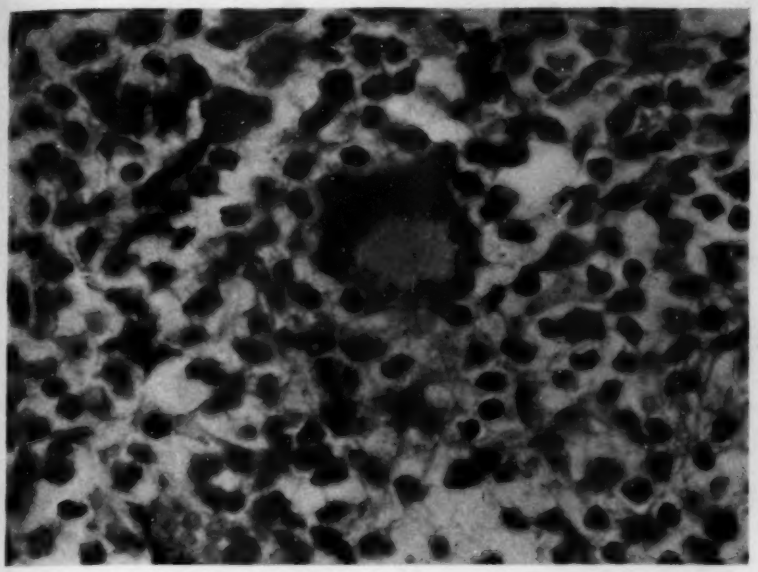

Fig. 7.—Langhans type of giant cell among perivascularly situated histiocytes in a sensitized calf five days after injection of tuberculin; × 800.

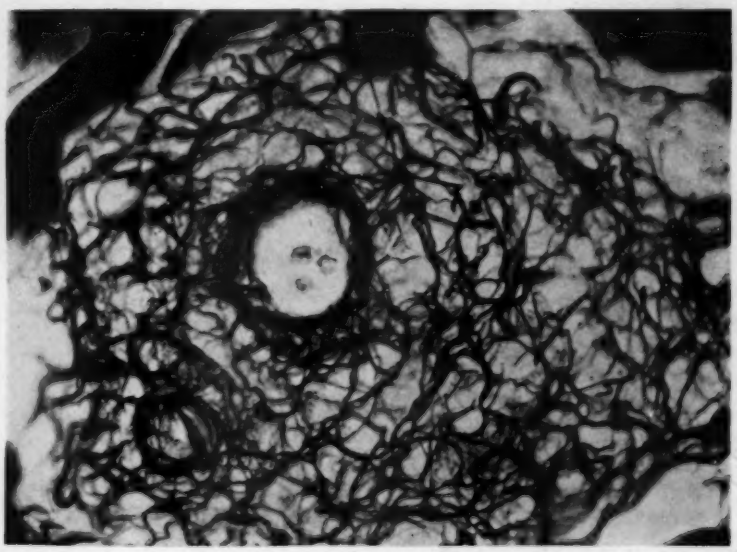

Fig. 8.—Reticulum fibrils in a perivascular cellular focus in a sensitized calf ten days after injection of tuberculin; × 440.

Fig. 9.—Cross-section of the entire dermis showing extensive cellular reaction in the reticular zone in a sensitized calf fourteen days after injection of tuberculin; × 6.

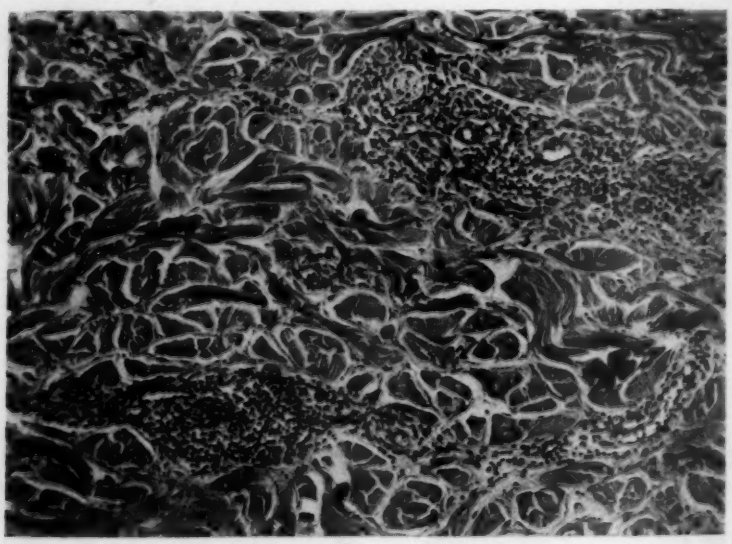

Fig. 10.—Perivascular and perineural accumulations of histiocytes in the reticular zone of the dermis of a tuberculous calf twenty-eight days after injection of tuberculin; × 100.

Fourteenth Day.—The perivascular and perineural cellular accumulations remained (fig. 9). The edema had disappeared, and the cells in the areas of the reaction gave evidence of condensation. Although the majority were adult histiocytic forms, a few lymphocytes and many eosinophilic granulocytes were present. The cytoplasm of the histiocytes had a clear, unstained appearance, and reticulum fibrils were exceedingly numerous. There were noted also a few giant cells of the Langhans type.

Twenty-First Day.—The caudal fold available for biopsy on the twenty-first day failed to give evidence of an appreciable macroscopic reaction, although in the opposite caudal fold there was a marked reaction. When the tissue was examined microscopically, significant changes were not observed.

Twenty-Eighth Day.—Many focal and diffuse accumulations of cells, predominantly histiocytic, were present in the papillary and reticular zones of the dermis (fig. 10). Organized thrombi were noted. In general, however, the reaction appeared to have reached a stage of quiescence.

Controls.—In the tissues removed from the two control, or nontuberculous, calves significant histologic alterations were not found. The last specimen taken for biopsy from these calves was obtained seventy-two hours after the administration of tuberculin (fig. 6 *B*).

COMMENT

A perivascular and perineural cellular response which at first is predominantly polymorphonuclear but which gradually becomes histiocytic seems to distinguish the reaction of the allergic, or sensitized, tissues of calves to tuberculin. Marked or destructive necrosis or extensive hemorrhage apparently does not occur, although endovascular changes are observed sufficiently often to seem of significance. The formation of thrombi, the extensive occurrence of edema, the progressive character of the changes in the intimal layer of some arteries and the intense focal accumulations of leukocytes are criteria of a vigorous, if not a violent, reaction. The fact, however, that necrosis is minimal indicates that the provocative factor in the genesis of the process has propensities which stimulate a cellular reaction rather than destruction or necrosis of tissue.

In the experimental material examined, giant cells of the Langhans type were not observed in the reactive process prior to five days subsequent to the injection of tuberculin. However, in material obtained for biopsy from spontaneously infected cattle, giant cells were seen frequently seventy-two hours after injection of tuberculin. Stewart and Rhoads [6] considered the giant cells of the intradermic tuberculin reaction of no immunologic significance but rather as evidence of the development of a nonspecific tissue reaction in response to necrosis. The fact that the necrosis present in the tissues examined was minimal may explain the paucity of giant cells in our material.

6. Stewart, F. W., and Rhoads, C. P.: Am. J. Path. **2**:571, 1926.

That the cellular reaction persisted so long was somewhat surprising and was probably attributable to the character of the cellular response. Because the response is largely histiocytic after the first few hours, and because there is but slight tendency for necrobiosis to occur, many of the cells and the foci they form apparently can continue in the tissue of the dermis for a prolonged period. Although a few instances were observed in which some of the histiocytes seemed to be assuming the character of fibroblasts, the evidence of this change was not convincing. Information concerning the ultimate cellular changes was not obtained from the material studied. From the data secured it seems that it would be necessary to follow the histology of the tuberculin reaction in calves longer than twenty-eight days in order to study the resolution of the reactive process.

The intracutaneous injection of tuberculin into sensitized bovines incites a rather definite and characteristic cellular response, which seems dependent for its appearance on the specific reaction of the allergic tissue cells to tuberculoproteins. Dienes and Mallory [7] and Laporte [8] expressed the belief that the resultant morphologic changes are definitely dissimilar to anaphylactic reactions of the derma and therefore constitute an important difference between these two forms of hypersensitiveness.

Our observations are not entirely in accord with those of Dienes and Mallory so far as the character of the cellular reaction during the first few hours of the process is concerned. While the initial cellular reaction in our material was predominantly polymorphonuclear, Dienes and Mallory working with sensitized guinea-pigs observed a well marked accumulation of large mononuclear cells, with practically no polymorphonuclears, as early as two hours after the injection of tuberculin. In the tissues we studied, polymorphonuclear leukocytes figured prominently in the regions of injection during the early phases of the reaction, but beyond the thirtieth hour there was a marked and constant diminution in the number of these cells, which was correlated with a gradual increase in the number of histiocytes. Dienes and Mallory found that the greatest number of histiocytes occurred during the earlier and later stages of the reaction, with the polymorphonuclear response most evident between the seventh and forty-eighth hours. They expressed the belief that the infiltration of polymorphonuclears during the intermediate phase of the process might be accounted for at least in part by the regions of necrosis in the epithelium.

Possible explanations for the differences in the cellular reactions in the material studied by Dienes and Mallory and in that studied by us

7. Dienes, L., and Mallory, T. B.: Am. J. Path. **8**:689, 1932.

8. Laporte, R.: Ann. Inst. Pasteur **53**:598, 1934.

include the species of animal sensitized, the degree of sensitivity at the time the tuberculin was injected and the amount of tuberculin used. Unless these factors are taken into consideration it seems unwise to compare the results of the two experiments.

SUMMARY

A histologic study was made of the tissue changes which follow intracutaneous injection of tuberculin into experimentally sensitized calves. The study included nine calves which were infected with bovine tubercle bacilli and two control calves which were not infected. After the lapse of fifty-eight days, the usual diagnostic dose of mammalian tuberculin was injected into the derma of each caudal fold of each calf. Starting at the third hour after the tuberculin was introduced, and continuing at intervals to the twenty-eighth day, portions of the respective caudal folds were removed for biopsy.

The essential histologic features can be summarized as follows: The reactive process gave evidence of a constant predilection for the perivascular and perineural tissues. During the early phases of the reactive process polymorphonuclear leukocytes were numerous. Eosinophilic granulocytes and histiocytes were in the minority. A histiocytic or mononuclear cellular reaction gradually replaced the polymorphonuclear leukocytes and dominated the picture, beginning at the sixtieth or the seventy-second hour. Edema appeared early in the reaction and disappeared between the fifth and seventh days. Certain endovascular changes, including thrombosis and endarteritis, occurred. Resolution of the cellular reaction had not occurred after twenty-eight days.

The injection of tuberculin into the skin of nonsensitized calves failed to provoke demonstrable changes.

CHOLESTEROL-INDUCED ARTERIOSCLEROSIS IN RABBITS, WITH VARIATIONS DUE TO ALTERED STATUS OF THYROID

FRANK R. MENNE, M.D.

JOSEPH A. P. BEEMAN, M.D.

AND

DANIEL H. LABBY, B.A.

PORTLAND, ORE.

We have made an attempt to study the effects of alterations in metabolism on the deposition of cholesterol in the arteries of rabbits, employing mainly alterations in thyroid function.

TECHNICAL PROCEDURE

Mature white, brown and gray chinchilla rabbits were divided into four groups of ten each. All of the animals were selected from supposedly healthy stock and were of fairly uniform weight but were purchased on the open market, so that their exact condition was not known. They were placed in individual cages and given a standard ration of commercial pellet food, green vegetables and water. After the blood cholesterol [1] and the basal metabolic rate of each had been determined,[2] the feeding of cholesterol was begun according to the method used by Leary.[3] Chemically pure cholesterol, repurified, was used in all of the experiments.

Oil feedings were given as follows: Five grams of cholesterol was dissolved in 100 cc. of olive oil U. S. P. on the water bath and the mixture placed in a thermos container. Twenty cubic centimeters of this mixture, at approximately body temperature, was injected under moderate pressure via a stomach tube. Coconut oil was occasionally used, but its high melting point made its use technically difficult. After feeding, the rabbits tended to vomit the mixture, with some evidences of gagging. It was also noted that the oil-fed animals lost weight and had diarrhea of an oily character. For these reasons, oil feeding of cholesterol was discontinued, and pellet feeding was instituted. The regular pellet food of the animals was made into a paste with water, and a weighed amount of cholesterol triturated; the mixture was divided into aliquot parts, containing 1 Gm. of cholesterol each, and small pellets were molded. These were then air dried at room temperature. After preliminary starving of the animals for a day, the pellets con-

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From the Department of Pathology of the University of Oregon Medical School.

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1. Sackett, Guy E.: J. Biol. Chem. **64**:203, 1925.
2. Stewart, J. D., and Menne, F. R.: Endocrinology **17**:93, 1933.
3. Leary, T.: Arch. Path. **17**:453, 1934; **21**:419 and 459, 1936.

taining cholesterol were placed in the food dish, and as soon as the rabbits had eaten them their regular food was added to the dish. Within three days they would eat the pellets without delay. No regurgitation or diarrhea was noted with this method.

Desiccated thyroid U. S. P. was incorporated in similar pellets and given in the same manner, the amount of thyroid in each pellet being known.

Iodine was at first administered via stomach tube as compound solution of iodine U. S. P.; later it was incorporated into the food pellets in a manner similar to that of thyroid and cholesterol.

Determination of Cholesterol.—Two-tenths cubic centimeter of blood was removed by syringe from the marginal ear vein and added to 10 cc. of a mixture of alcohol and ether (60 cc. of redistilled alcohol plus 40 cc. of redistilled ether of reagent quality) in a 15 cc. conical centrifuge tube. The resulting flocculum was agitated for thirty minutes and the mixture centrifugated at high speed. The supernatant portion of the alcohol-ether mixture was evaporated on the water bath to dryness and then extracted with three 2 cc. portions of chloroform of reagent quality. The chloroform solution was placed in a stoppered graduated cylinder and made up to 10 cc. with chloroform. Acetic anhydride and sulfuric acid were added, and the resulting blue-green color was compared colorimetrically with an aqueous solution of naphthol green B and methylene blue. Control determinations were made from time to time by adding cholesterol to blood of known cholesterol content.

Determination of the Basal Metabolic Rate.—The apparatus used consists of a sealed metal box with inlet and outlet tubes connected to a McKesson metabolor. The animals were given thirty minutes to become accustomed to the instrument before readings were taken. The usual technical precautions and standard calculations were employed.

RESULTS

Group A, the Control Group.—These rabbits had nothing but the standard diet and the continuous daily (?) administration of pure cholesterol in oil and in pellets over periods of from one hundred and one to one hundred and ninety-nine days (table 1). They received varying quantities of cholesterol in oil (from 69 to 128 Gm.) and in the form of pellets (from 19 to 28 Gm.) with a total intake of not less than 69 Gm. nor more than 147 Gm., so that most of the rabbits received more than 130 Gm. of cholesterol in addition to an abundant (ad libitum) diet. Determinations of the cholesterol in normal blood showed concentrations varying between 61.2 and 110 mg. per hundred cubic centimeters. The basal metabolic rates varied from plus 21 to plus 32, being fairly constantly in the range between plus 25 and plus 30. All of these rabbits gained weight during the period of feeding. Eight of them were killed at the end of the experiment, while two died of aspiration pneumonia. It is to be noted that six (60 per cent) of these rabbits showed atherosclerosis of the aorta. It was absent in those receiving under a total of 100 Gm. of cholesterol and was marked in those receiving a larger amount, with a consequent high value of blood cholesterol (210 to 510 mg.). Deposits of the lipoid were found most

TABLE 1.—*Data Concerning Rabbits Fed Cholesterol (1 Gm. Daily)—Control Group A*

Rabbit	Days	Cholesterol Feedings			Blood Cholesterol Variations, Mg.	Basal Metabolism, +	Fate of Animals	Weight, Kg.	Thyroid	Atherosclerosis		Organs Showing Cholesterosis	Comment
		In Oil, Gm.	In Pellets, Gm.	Total, Gm.						Gross	Microscopic		
1A	152	110	0	110	76.0	21-31	Killed	+1.5	Inactive	0	0	None	Hepatic lipoid 1
2A	101	69	0	69	70.8	29-30	Died	+1.5	Inactive	0	±	None	Hepatic lipoid 3
3A	102	70	0	70	61.2	26-32	Died	+2.0	Inactive	0	0	None	Hyperemia of lungs
4A	192	119	25	144	70.8-210	29-30	Killed	+1.8	Inactive	±	1+	None	Fungoid infection of ear, fall in weight
5A	199	119	28	147	294-300	26-31	Killed	+2.2	Inactive	±	0	None	Variation in weight due to diarrhea
7A	183	118	19	137	208-324	27-33	Killed	+2.5	Inactive	1+	1+	None	Hepatic fat 2, diarrhea
8A	196	128	19	147	91-500	25-29	Killed	+2.4	Inactive	4+	4+	Ovaries, adrenals	Hepatic lipoid 2
9A	176	119	28	147	88-510	29-32	Killed	+1.7	Inactive	4+	4+	Adrenal, corneal opacity	Hepatic fat 2, lipoid, diarrhea
10A	175	113	28	141	86.9-314	28-30	Killed	+1.7	Inactive	4+	4+	Adrenals	Hepatic lipid 2

frequently in the adrenal glands; they were less abundant and less apparent in the other organs. In every one of these rabbits the liver presented evidence of an excess of lipoid in the cells, which were swollen and of the clear cell type. In the rabbits that died of pneumonia, cholesterol was found in the bronchi. This was due either to

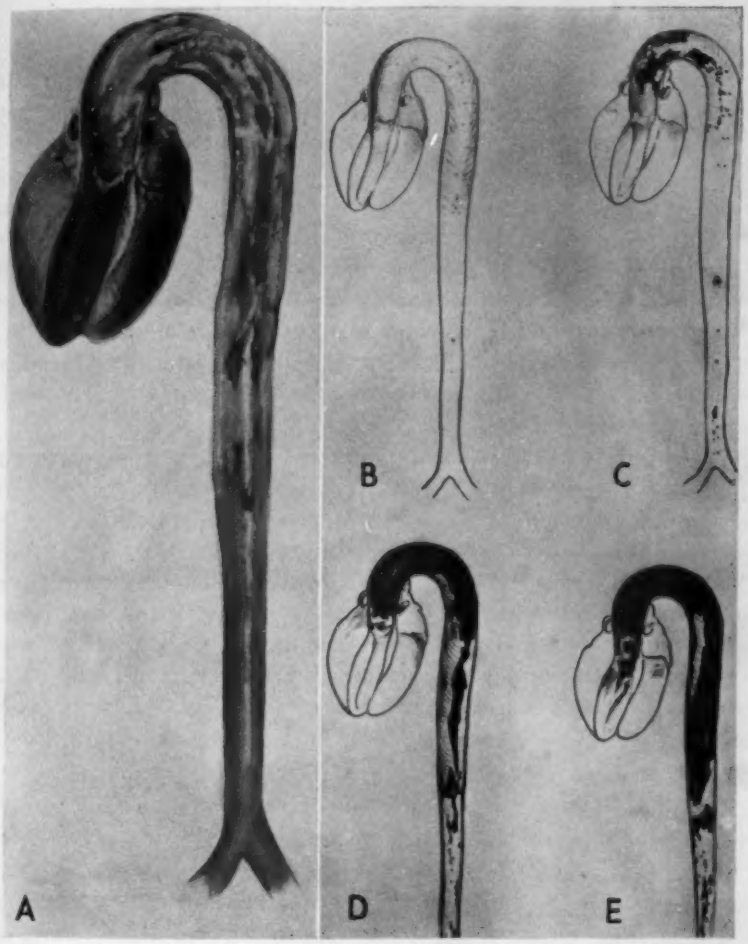

Fig. 1.—Sketches illustrating the extent, distribution and character of the induced atherosclerosis.

an error in the technic of administration or to regurgitation and aspiration. Careful search failed to disclose any evidence of depositions (atherosclerosis) in smaller arteries in the various organs and tissues of these animals.

The gross aspects of the atherosclerotic changes were characteristic; the changes were confined to the aorta and distributed according to the usual occurrence of such lesions in both man and animals (fig. 1). The extent of such lipoid changes was not always proportionate to the degree of cholesterosis. None of these areas of fatty change exhibited gross evidence of necrosis or of deposition of lime salts, probably because of the failure of absorption and the diarrhea early in the experiment.

Microscopic examination revealed all stages of cholesterol deposition in the aorta. Even when there were no visible impregnations in the aorta occasional groups of monophages (lipophages) containing a few vacuoles could be seen lying juxtaposed to the intima, as if glued to its surface, in the depression of an intimal fold. In some instances there were larger aggregations of the foam cell type with alteration of the underlying intima and subintimal palisading of cells. The latter pattern was prominent in all phases. In the progression of the process such accumulations of lipoid-laden phagocytes were found either in the form of subendothelial hillocks or as diffuse intra-intimal and subendothelial blankets. The manner in which the endothelial lining was penetrated was not clear.

It seemed from the study of different sections that subsequent to a piling up of phagocytosed cholesterol necrobiosis of the underlying endothelium occurred, with defensive palisading of fibroblasts. Subsequent to this the endothelium evidently grew over the top of the mass. From then on the appearance was that of continuous infiltration with encroachment on the media. The outer border of dissemination in the aortic wall was often irregular and not unlike the jagged fat lines so frequently seen subepicardially in fatty hearts. The appearance of these invasions indicated drainage (lymphatic ?) through the aortic walls in the direction of the adventitia, with later necrosis and fibrosis (figs. 1 and 2).

Group B.—Thyroidectomies were performed under ether anesthesia on all of the ten rabbits. Considerable difficulties were experienced in getting the animals to survive the operations, especially during the summer months. Accidental destruction of the recurrent laryngeal nerve together with other traumatism was followed by immediate death after the operation or later from respiratory infections. The mortality rate under the best of conditions was about 60 per cent. Four of the animals lived long enough (from seventy-nine to one hundred days) to consume from 71 to 92 Gm. of cholesterol, with production of cholesteremia of from 93 to 410 mg. per hundred cubic centimeters and an incidence of atherosclerosis of the aorta of varying degrees in 75 per cent. Following the removal of the thyroid there was an appreciable drop in the basal metabolic rate, ranging downward from 21 to 13 per cent, with a

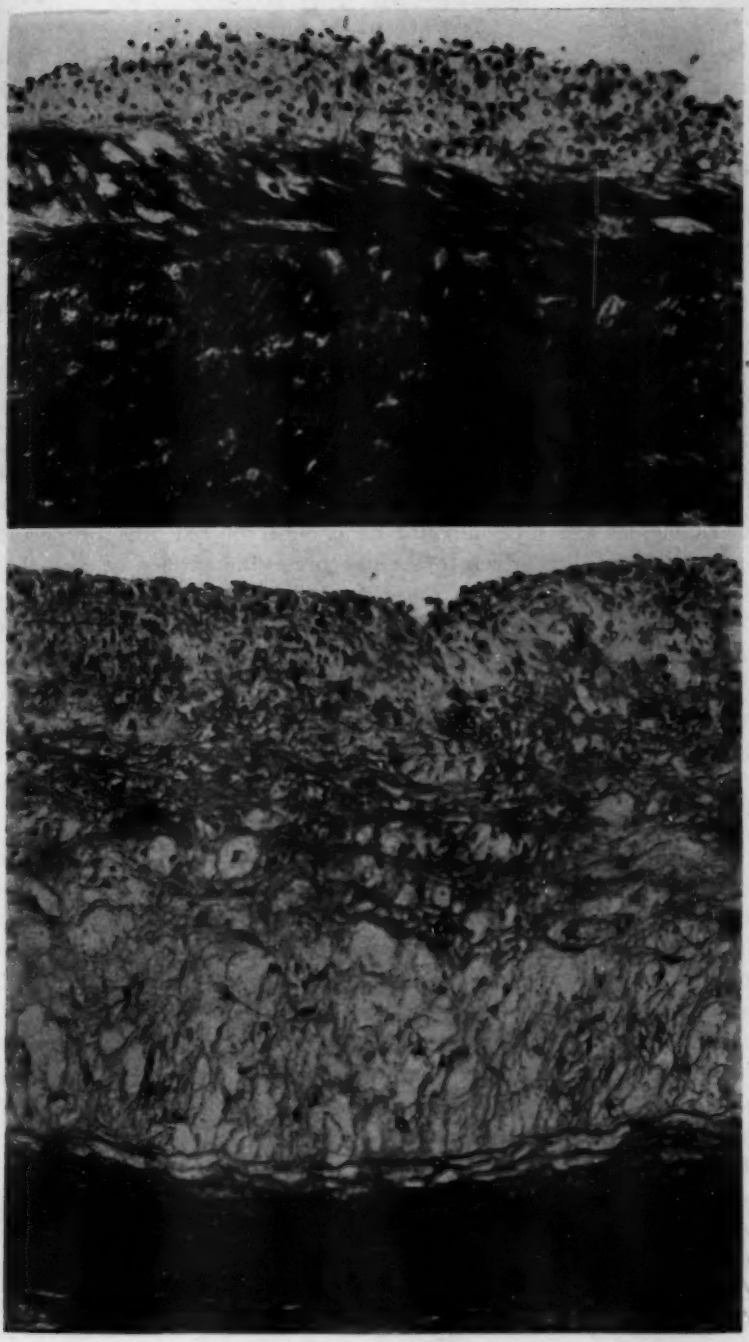

Fig. 2.—Photomicrographs illustrating (upper) a moderately early deposition of lipoblasts in the intima and (lower) an advanced infiltration into the media, the lamellae of which are being spread apart.

moderate increase in weight (after a sharp postoperative fall) and a partial terminal diminution due to pulmonary infections and diarrhea. It is apparent that the removal of the thyroid and the consequent slowing of catabolism resulted in hypercholesteremia with a smaller intake, followed by a greater incidence of atherosclerosis (75 per cent) and much more extensive atherosclerosis over a shorter period of time. The gross and microscopic changes were similar to those observed in the control animals (group A, table 1).

Subgroup B.—Five rabbits were given compound solution of iodine in quantities ranging from 60 to 210 cc. over periods of from sixty-one to eighty-seven days. Cholesterol was simultaneously fed in amounts of from 50 to 72 Gm. each. The cholesterol content of the blood increased up to from 301 to 333 mg. per hundred cubic centimeters; the basal metabolic rate was decreased, and the atherosclerosis was found to be marked in three of the animals that survived. This incidence is remarkable, as the total amount of cholesterol fed was not great in comparison with that given to the two preceding groups of animals. It is also noteworthy that the thyroid administered reduced the activity of the thyroid gland, as was evidenced in its microscopic appearance and the corresponding drop in the basal metabolic rate. In one instance (15B), however, there was evidence of increased activity of the thyroid gland, with possible influence on the extent of the atherosclerosis produced. It was therefore apparent that the administration of iodine had an effect similar to that of the removal of the gland in the animals of the previous group (B), which experiment it supported.

Group C.—All the animals were fed cholesterol in similar daily doses for periods ranging from one hundred and twenty-two to one hundred and ninety-nine days, with one exception (7C, fifty-eight days; table 4). During this period a total of from 104 to 147 Gm. of cholesterol was fed with two exceptions (7C and 8C received 32 and 86 Gm., respectively). During the entire time each animal was given varying doses, from 0.1 to 0.5 Gm., of desiccated thyroid, which was also mixed with the pellets. The amounts were gradually increased until the rabbits exhibited marked loss of weight, cachexia and a tendency toward diarrhea and increased irritability. The amount of desiccated thyroid was then reduced, and the animals were allowed to recover to within the range of their normal state, when they were again fed an increased quantity of desiccated thyroid to the point of recurrence of the symptoms of hyperthyroidism, so that all these ten animals passed through periods of increased and decreased metabolism, as is shown by the figures in the weight column. During this period the basal metabolic rates ranged upward from 2 to 3 degrees, and in one instance (9C), 14 degrees. The variations in basal metabolic rates corresponded to the

TABLE 2.—*Data Concerning Rabbits Fed Cholesterol (1 Gm. Daily) and Thyroidectomized to Reduce Metabolism—Group B* *

Rabbit	Days After Thyroidectomy	Cholesterol Feedings			Blood Cholesterol Variations, Mg.	Basal Metabolism, +	Fate of Rabbits	Weight, Kg.	Atherosclerosis		Cholesterosis	Comment
		In Oil, Gm.	In Pellets, Gm.	Total, Gm.					Gross	Microscopic		
2B	100	50	0	50	80-93	31-18	Died	+0.5	0	0	0	Pneumonia
3B	105	87	0	87	325-333	21-16	Killed	+1	3+	3+	Liver engorged	Trace of thyroid remaining
4B	79	71	0	71	325-384	16-13	Killed	+1	2+	2+	Adrenal	No thyroid present
5B	100	92	0	92	410	19	Died	±	4+	4+	0	Terminal pneumonia; gradual decrease in weight due to infection

* Six rabbits died immediately after operation from various causes.

TABLE 3.—*Data Concerning Nonthyroidectomized Rabbits Fed Cholesterol and Subsequently Given Iodine in Varying Doses—Subgroup* B

Rabbit	Days	Compound Solution of Iodine, Cc.	Cholesterol Feedings			Blood Cholesterol Variations, Mg.	Basal Metabolism, +	Fate of Animals	Weight, Kg.	Atherosclerosis		Cholesterosis	Comment
			In Oil, Gm.	In Pellets, Gm.	Total, Gm.					Gross	Microscopic		
11B	61	60	44	6	50	0	0	Died	+0.1	0	0	0	Aspiration pneumonia
12B	61	60	44	6	50	0	0	Died	—1.0	0	0	0	Bronchopneumonia
13B	87	210	44	28	72	263-329	18.8	Killed	+1.0	4+	4+	0	Coccidiosis
14B	87	210	44	28	72	317-333	22.2	Killed	+0.5	2+	2+	0	Huge distention of thyroid acini
15B	87	210	44	28	72	242-301	20.8	Killed	+1.5	3+	2+	0	Hyperplasia of thyroid with diminution in colloid content

degrees of toxicity existing in the different animals at the time the determinations were made. The blood cholesterol values had a tendency to remain around normal, with only a slight elevation during the periods of reduction of the hyperthyroidism, when the dosage was decreased. It will be noted that in only four of the rabbits was there an elevation of the percentage of cholesterol in the blood. Because of the rigorous induction of hyperthyroidism, all but two of these animals were found dead. Postmortem examinations revealed evidence of atherosclerosis only in rabbit 9C, in which there was a limited spotty deposition in the aorta. One other rabbit (3C) disclosed early microscopic deposition of lipoblasts.

Most of these animals were greatly emaciated, with evidence of diarrhea. In several of them cholesterol was found in the bronchi in association with terminal bronchopneumonia. In one there was marked fatty degeneration of the liver, and in two others there was marked fibrosis of the myocardium similar to that described by us [4] in a report on the myocardium in hyperthyroidism. In most of the instances the thyroid gland was found to be histologically normal. The results in this experiment indicate that administration of amounts of desiccated thyroid adequate to elevate the basal metabolic rate to the level observed in hyperthyroidism resulted in complete metabolism of the excess amount of cholesterol fed and prevention or removal of deposits in the tissues. The liver, as well as the other organs and tissues, disclosed inanition atrophy.

COMMENT

The administration of cholesterol to rabbits is not without difficulties, which undoubtedly give rise to variation in the results obtained by us as well as others. The passage of the stomach tube for the administration of an oily substance may result in introduction or escape of some of this substance into the air passages, with respiratory disturbances, while from the excess of oil in the stomach, diarrhea may result with not only reduction in absorption but also loss of water and nutrition, tending to increase the metabolism. These factors are especially significant because of the duration of the experiment. The method used by Leary [3] was not altogether satisfactory for the rabbits used by us. We finally resorted to pellet feeding, with better and more constant results.

While there is manifest constancy in the relationship between supersaturation of the blood with cholesterol and production of atherosclerosis, it was not possible for us to control the amount of this lipoid in the blood with the degree of accuracy reported by some of the inves-

4. Menne, F. R., and others: Arch. Path. **17**:333, 1934.

TABLE 4.—*Rabbits Fed Cholesterol and Desiccated Thyroid—Group C*

Rabbit	Days	Cholesterol Feedings			Desiccated Thyroid, Gm.		Blood Cholesterol Variations, Mg.	Basal Metabolism, +	Weight, Kg.	Fate of Animals	Atherosclerosis		Comment
		In Oil, Gm.	In Pellets, Gm.	Total, Gm.	Daily	Total					Gross	Microscopic	
1C	170	119	6	125	0.1-0.5	38.2	74.2(?)	29-34	1.87 3.5 2.7	Died	0	0	Cholesterol in bronchi; terminal pneumonia
2C	199	119	28	147	0.1-0.5	38.0	70.8-281.2	26-31	2.07 3.5 1.77	Died	0	0	Slight hyperplasia of thyroid; terminal pneumonia
3C	152	104	0	104	0.1-0.5	20.9	91.2	26-28	2.1 4.1 3.1	Died	0	2+	Fatty degeneration of liver
4C	199	119	28	147	0.1-0.5	38	83.1-280.3	26	2.1 3.6 2.3	Died	0	0	Terminal pneumonia
5C	175	119	11	130	0.1-0.5	22	87.1	28-28	2.0 4.5 2.0	Died	0	0	Terminal bronchopneumonia; cholesterol in bronchi
6C	199	119	28	147	0.1-0.5	38	70.2-292	26-28	2.0 3.7 2.4	Died	0	0	Slight hyperplasia of thyroid; emaciation
7C	58	32	0	32	0.1-0.2	1.3	81.3	29	1.87 2.0	Died	0	0	Fibrosis in media and myocardium
8C	122	86	0	86	0.1-0.5	16.4	79.7	28.5-30	2.3 3.0	Died	0	+	Terminal pneumonia; fibrosis of heart
9C	199	119	28	147	0.1-0.5 (0.25 Gm. for 3 doses)	38	75.7-294	26-40	1.8 3.5 3.0	Killed	2+	2+	Fibrosis of myocardium and of media of aorta; inactivity of thyroid
10C	199	119	28	147	0.1-0.5	38.2	83.1-290	28	2.0 3.3 1.0	Killed	+	+	Inactivity of thyroid

tigators (Turner [5]). Even when lesions are so produced, the question arises as to their significance in regard to human arteriosclerosis. Leary [3] listed the main criticisms advanced by others against the use of rabbits for experiments in producing atherosclerosis with cholesterol. Among these contentions it is pointed out that the rabbit with its herbivorous diet does not normally consume cholesterol. However, Rosenthal [6] observed that cholesterol is normally found in the rabbit's blood, coming from both endogenous and exogenous sources, an observation which has repeatedly received support from others' observations, including our own. Naturally the normal percentage of cholesterol in the blood is lower in rabbits than in man. Although there is this difference in the rabbit, the factors which control the abnormal amounts of cholesterol in the blood as listed by Herxthal and Hunt [7] probably operate in the rabbit as well as in man and are worthy of consideration. These authors regard excess accumulations as due to (1) abnormal synthesis, (2) tissue affinity and retention, (3) abnormal precipitation or liberation, (4) hemoconcentration or dilution and (5) failure of destruction or elimination. It is therefore natural that there should be variations in the deposition of cholesterol in different rabbits as there is in man, even though the amounts ingested are comparable. Such irregularities in the production of atherosclerosis were observed by us in animals receiving the same dose of cholesterol per unit of body weight over the same period of time.

Regardless of the differences of opinion as to the sources of cholesterol in herbivorous and omnivorous animals and the variations in results, the fact that the atheromatous lesions of arteries always contain cholesterol stands out in bold relief. This is supported by numerous studies.[8] There seems, therefore, to be no room for doubt as to the occurrence of hypercholesteremia in proportion to the extent of the atherosclerotic lesions of man as he advances in age and the comparability of this process with that seen in experimental animals.

The disagreements between the investigators as to the significance of cholesterol-induced atherosclerosis result from differences in their concepts of its participation in the pathogenesis of such lesions. Rabinowitch [9] in calling attention to the frequency of an increase in the cholesterol of the blood plasma in alcoholism, nephrosis, gout, pregnancy, myxedema, biliary obstruction and diabetes points out that there may not necessarily be an increase but an alteration in the ratio between free

5. Turner, B.: J. Exper. Med. **58**:115, 1933; **62**:721, 1935.
6. Rosenthal, S. R.: Arch. Path. **18**:473, 1934.
7. Herxthal, F. M., and Hunt, H. M.: Ann. Int. Med. **9**:717, 1935.
8. Zech, B. M.: Am. J. Path. **12**:115, 1936. Windaus, A.: Ztschr. f. physiol. Chem. **12**:115, 1936. Rosenthal.[6]
9. Rabinowitch, I. M.: Ann. Int. Med. **8**:1436, 1935.

cholesterol and cholesterol esters. Any condition, therefore, which lowers the metabolism (particularly of carbohydrates) in man (various diseases) or in animals (thyroidectomy and administration of iodine) will result in abnormally increased amounts or altered states of the cholesterol in the blood and further in its deposition. On the other hand, human diseases such as febrile disorders, hepatitis, severe anemia, syphilis, tuberculosis and hyperthyroidism result in a decrease or alteration of the state of the cholesterol in the blood and the absence or retrogression of arteriosclerosis. These observations are supported by many studies of human and experimental material. Accordingly, a consideration of the development of the lesions of senile arteriosclerosis must assume the existence of an alteration in, or an increase of, cholesterol in the blood and an increase in the permeability of the endothelial lining and in the receptability of the subintimal ground substance to lipoid deposits. Most important in the development of hypercholesteremia are conditions which lead to great reduction in the metabolism of cholesterol, since atherosclerosis will apparently not occur, regardless of the altered condition of the wall of the blood vessel, if the level of the cholesterol in the blood is kept low, as in wasting diseases in man and induced hyperthyroid states in rabbits.

Not only do the various disease processes mentioned alter the arterial wall, but their effect is markedly modified by tension and other mechanical factors resulting from variation in caliber and mechanical traumas secondary to function. The location of atherosclerosis at points of angulation or of changes in the direction of current, i. e., in recesses where swirls and eddies of the blood stream naturally occur, such as the anterior leaflet of the mitral valve, the sinus of Valsalva, the isthmus of the aorta and the mouths of the intercostal and other arteries leaving the aorta, as well as the linear distribution, all attest the augmenting influence of the mechanics of the circulation which obtains in both human and experimental arteriosclerosis. Duguid [10] and others are of the opinion that atheroma of the aorta is a condition common to more than one type of aortic disease. It is contended by him that the increased thickness of the intima in adult life, the local traumatic destruction resulting from the elasticity produced by the "drag effect" of vessels leaving at right angles, and the folds formed in the intima by the diastolic recoil are all operative in producing cholesterol deposits. Once the deposition is started reenforcement fibrosis, later degeneration from altered nutrition, further loss of elasticity and necrosis from lack of nutrition, with final calcification, occur. We agree with Krafka [11] that the histogenesis of both human and experimental atherosclerosis in part supports this contention. Certainly an increased pulse rate,

10. Duguid, J. B.: J. Path. & Bact. **29**:371, 1926.
11. Krafka, J.: Arch. Path. **23**:1, 1937.

hypertension and other clinical conditions producing additional strain tend to yield a greater incidence of such lesions in the aorta as compared with smaller arteries (Rosenthal [6]), in which support from extravascular tissue is greater than it is in the aorta and the latter's larger branches, and in which internal pressure is low and the drag of branching vessels is not important. Harrison,[12] in further support of the mechanical theory advanced by Duguid, suggested from his experiments with cholesterol-fed rabbits, in which vitamin D-induced sclerosis was added to that from cholesterol, that both of these lesions occur on a mechanical basis.

Accordingly there seems to be a preponderance of evidence from the studies of atherosclerosis in human beings from clinical and pathologic points of view, as well as from the production of similar lesions by cholesterol feeding, that the essential factor in the production of this disease of the blood vessels is the occurrence in the blood stream of a variable amount of cholesterol, either in the pure form or as esters, and that the presence of this lipoid predisposes to atherosclerosis in proportion to the approach to saturation of the blood with this lipoid. This seems true since atherosclerosis does not develop in those diseases which tend to deplete the blood stream of its cholesterol, conditions which are comparable to controlled experiments in rabbits fed cholesterol under conditions of increased metabolism. It seems to us that the presence of varying amounts of cholesterol or of its esters in the blood stream and the alteration due to the mechanics of the circulation are the two agencies initiating atherosclerosis. Once started the filtration of the substance into the tissue spaces and lymphatics of the vessel wall and its accumulation there undoubtedly serve to produce a vicious cycle of destruction absorption and defensive regeneration.

Despite all criticisms to the contrary it seems to us that the contentions of Leary are tenable. In our considerations it seems evident that many agencies of disease, the variations in diet, the disturbance of internal secretions, infections, the traumatism of certain occupations and the influence of posture all aid in the development of arteriosclerosis only as they modify these two basic factors of hypercholesteremia and mechanical strain.

SUMMARY

Rabbits fed pure cholesterol in oil and pellets over a long period under different conditions, namely, (*a*) under normal conditions (controls), (*b*) following thyroidectomy and reduction in metabolism, as well as after administration of iodine to inhibit thyroid function, and

12. Harrison, C. V.: J. Path. & Bact. **36**:446, 1933.

(*c*) during administration of desiccated thyroid in such a manner as to produce intermittent periods of progression and regression in metabolism, all acquired atherosclerosis in varying degrees. In the rabbits under normal conditions but to a greater extent in the rabbits with a depressed metabolic rate (due to removal of the thyroid or to administration of compound solution of iodine) the production of such lesions was readily accomplished. We are led to conclude that our results tend to corroborate the major contention of Leary that there are two primary conditions necessary to the development of atherosclerosis, (*a*) an excess of cholesterol or of cholesterol esters in the blood and (*b*) the stress due to mechanical factors of the circulation.

Case Reports

LEUKEMIC RETICULO-ENDOTHELIOSIS

Report of a Case with Autopsy

C. B. Wright, M.D., and E. H. Norris, M.D., Minneapolis

Through the addition to the literature of reports on carefully studied cases, notions of the reticulo-endothelial system and its reactions in disease are slowly taking on better form. However, the remarkable reactivity of the reticulo-endothelium results in a variety of anatomic changes which have been variously interpreted. Not only has the interpretation of lesions varied but, through the efforts of investigators to impose their views, a confusing descriptive terminology has been created. Ultimately, after more cases have been thoroughly studied, an analytic attack may be made on the problem to the end of bringing order out of the present confusion.

Any one who considers the pathologic changes of the reticulo-endothelial system encounters the problem of distinguishing between neoplasia and hyperplasia. One is also brought face to face with the necessity of setting limits on effects produced by the action of known etiologic agents. Finally, certain of the problems concerned with leukemia and the manifestations in the circulating blood of changes in the reticulo-endothelial system are involved. Opinions regarding the etiology of reticulo-endotheliosis are various. For these reasons it is not strange that the nature of this malady should be ill understood and that exact definition of the disease should still be wanting. Downey [1] has pioneered in this field, and his thoroughly fundamental research has opened paths which lead to the best interpretive concepts. We have accepted the term "leukemic reticulo-endotheliosis" in our presentation of a case because this name seems to cover the features exemplified in the tissues and because the term does not seem to involve us in the disputed questions implied by certain other terms which the literature contains—"lipoid histiocytosis," "nonlipoid histiocytosis," "aleukemic reticulosis," and the like. For example, Foot and Olcott [2] proposed the term "nonlipoid histiocytosis" in their effort to subdivide reticulo-endotheliosis. We have recently seen a case of

From the Departments of Medicine and Pathology, University of Minnesota.

1. Downey, H., in Bell, E. T.: Text Book of Pathology, ed. 2, Philadelphia, Lea & Febiger, 1934, p. 723.

2. Foot, N. C., and Olcott, C. T.: Am. J. Path. **10**:81, 1934.

Schüller-Christian disease in which the tissues showed some of the proliferating reticulo-endothelial cells containing lipoid and some without any lipoid. In other words, in the same case there were presented examples of the changes described in lipoid histiocytosis and of those described in nonlipoid histiocytosis. There seems little to be gained, therefore, through the multiplication of terms in the present state of knowledge. The case which we are about to describe presents a number of features that seem of importance and may aid somewhat in the understanding of this general group of diseases.

REPORT OF CASE

Summary of the Clinical Observations.—The patient was a white man aged 56 years, a retired business man, married. His wife was living and well and there were two grown children, quite healthy. His father died at the age of 60; the cause of death was unknown. The mother was living and well at the age of 78; one sister was living and well at the age of 50. There was no history of a disease of the blood in the family.

The patient had always been healthy. He had had the usual childhood diseases but never any severe illness. Eight years prior to his death a carcinoma had been removed from his lower lip and a block dissection of the neck done; this was followed by radiotherapy, and there had been no evidence of recurrence of the growth. The patient was considerably addicted to the consumption of alcoholic beverages, mostly whisky.

In May 1936 he noticed bluish spots on his skin. Shortly after these appeared, he suffered a sudden weak spell and became very pale; he had no pain but was dizzy at times. He was admitted to the hospital on June 30, where the following observations were made:

There were many purpuric spots scattered over the skin of the entire body; the skin and mucous membranes were pale; the heart and lungs and the blood pressure were normal; the liver and spleen were not palpable; the temperature was 98.6 F. on admission and remained normal except following transfusions. Occult blood was found in several examinations of the stools.

The summarized examinations of the blood yielded the following data: During about two weeks the hemoglobin dropped from 43 to 17 per cent, and the red blood cells, from 2,220,000 to 1,050,000. There were considerable anisocytosis, moderate poikilocytosis and slight polychromasia. From 2 to 4 nucleated red cells were found per hundred leukocytes counted. The blood platelets varied from 21,000 to 46,000. The clotting and bleeding time was six minutes. There was persistent leukopenia, and the white cells varied between 3,200 and 1,600 per cubic millimeter. Two differential counts (Dr. O. P. Jones) were as follows:

	June 30, 1936	July 3, 1936
Basophils	0	1.0
Eosinophils	0	1.0
Nonsegmented neutrophils (band forms)	17.0	7.5
Segmented neutrophils	36.5	12.0
Lymphocytes	29.0	32.0
Monocytes	5.5	6.5
Atypical reticulo-endothelial cells	12.0	40.0

Dr. Jones reported: "The cells classified as atypical reticulo-endothelial cells for the most part represent various intermediate stages of differentiation between reticulum and myeloblast forms. In a few instances the reticular cells seemed to be differentiating toward young lymphocytes."

"The cells in the blood smears on which the diagnosis of leukemic reticulo-endotheliosis was made may be described as follows (fig. 1*A*): The cytoplasm is moderate in amount, extremely basophilic but not homogeneous. Small areas of yellowish hyaloplasm embedded in the cytoplasm give it a mottled appearance. Dark azurophilic granulation is present in some cells. The nuclear structure approximates that of a myeloblast but on the whole is somewhat coarser. The strands of the chromatin network are thicker and stain darker than those of a true myeloblast. Definite areas of acidophilic parachromatin give the nucleus a sievelike appearance. The nucleoli are large, indistinct and irregular in outline."

The patient responded to no therapeutic measure, had several severe nosebleeds and died on July 17, after an illness of about two months. Four direct transfusions were given; two of these were of polycythemic blood. Calcium lactate, parathyroid, liver extract and bone marrow were also given.

Autopsy.—The body was that of a well developed, well nourished white man, 176 cm. in length and weighing about 160 pounds (72 Kg.). The body was embalmed. Hypostasis was evident over the posterior parts of the body. There was no edema, cyanosis or jaundice. There were numerous purplish subcutaneous ecchymoses from 1 to 3 cm. in diameter, distributed over both the lower extremities.

The peritoneal cavity contained no excess fluid and showed no adhesions. The diaphragm reached to the fourth rib on each side. The edge of the liver corresponded to the costal margin. The appendix was atrophic and lay free in the pelvis. The pleural cavities showed no excess fluid and no adhesions. The pericardial sac contained about 10 cc. of clear fluid; there were numerous petechial hemorrhages scattered over the epicardium.

The heart weighed 450 Gm. The valvular and mural endocardium showed no gross evidence of disease. The myocardium was normal in consistence, brown and showed no fibrosis. The aorta showed arteriosclerosis of grade 1. The coronary vessels were patent at their orifices and showed atherosclerosis of about grade 1 along their trunks.

The right lung weighed 750 Gm.; the left, 700 Gm. There was crepitation throughout, and no excess fluid and no pus could be expressed from either.

The spleen weighed 350 Gm. The capsule was tense and the edges rounded. On section, the pulp was soft, and no definite malpighian corpuscles could be recognized.

The liver weighed 1,200 Gm.; its surface was smooth. There was a long tonguelike extension of the left lobe, measuring 25 cm. in length by 10 cm. in width by 2.5 cm. in thickness, which was spread out over the left diaphragmatic dome. The cut surface of the liver showed no disease. The gallbladder was distended with about 60 cc. of thin dark bile; there were no concretions.

The stomach contained about 500 cc. of clotted blood; blood was also present in several loops of the small bowel and in the colon. There were large numbers of small diverticula of the large bowel from the cecum to the rectum. There were no other gross lesions in the gastro-intestinal tract.

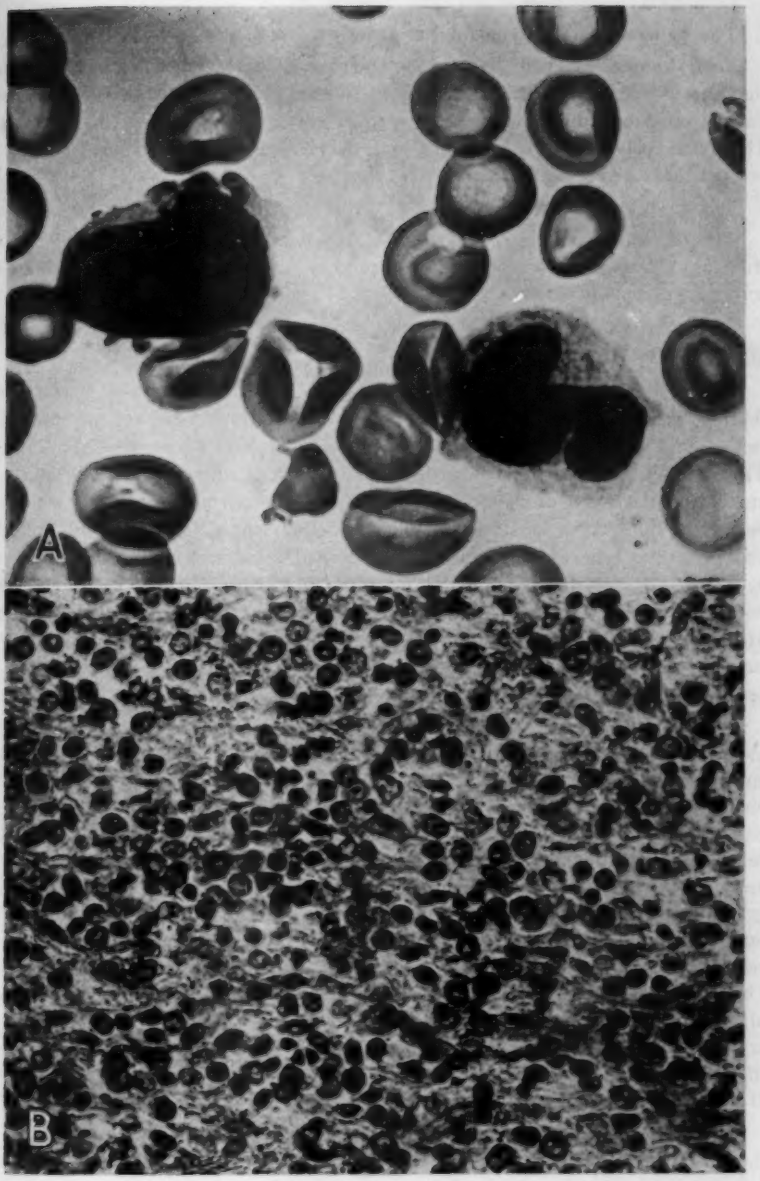

Fig. 1.—*A*, oil immersion photomicrograph of a field from a blood smear made on June 30, 1936. In the lower right hand corner is a normal monocyte. In the upper left hand corner is a monocytoid cell from the reticulum; the finding of this type of cell in the circulating blood made possible the correct antemortem diagnosis. *B*, medium power photomicrograph of the spleen. The large number of proliferating reticulo-endothelial cells in the sinusoids and pulp cords may be seen. Relatively few lymphocytes are found. Much granular pigment is present.

The pancreas and adrenals were grossly normal.

Each kidney weighed 180 Gm. Their capsules stripped with ease, leaving smooth surface. The cortex measured 8 mm. in thickness and was well differentiated from the medulla. There was a small cyst in the cortex of the right kidney. The renal pelves and the ureters and bladder showed no gross lesions. The prostate was remarkably small.

The thyroid and parathyroids were grossly normal.

The mesenteric and periaortic lymph nodes were not enlarged; they measured from 5 to 8 mm. in diameter. The bronchial and mediastinal nodes showed no enlargement but were black.

The marrow of the ribs and vertebral bodies was gray-pink and appeared grossly normal.

Microscopic Observations.—The stomach, small intestine, appendix, colon, thyroid, parathyroids, pancreas, heart, lungs and adrenals showed no microscopic evidence of disease. The kidney was normal except for two tiny cortical cysts. There was moderate atherosclerosis of the aorta, with considerable thickening and fatty infiltration of the intima. The liver was normal except for a moderate periportal infiltration of small lymphocytes. Sections of a number of mesenteric and periaortic lymp nodes showed quite normal structure, as did the solitary and aggregated lymph follicles of the intestine. One bronchial node showed much anthracosis and moderate hyperplasia of the reticulum such as is seen in conditions of chronic lymphadenitis; a few giant cells were seen in the sinuses of this node. Sections and imprints of the marrow from ribs and vertebral bodies showed no recognizable hyperplasia of the reticulum.

In the spleen there was marked hyperplasia of the reticulum. The pulp and most of the sinusoids were filled with large cells derived from the reticulo-endothelium (figs. 1 *B* and 2). These cells had large round or irregularly ovoid nuclei, which were outlined by heavy nuclear walls. There was moderate variation in the size of the nuclei. The nuclei appeared vesicular because of the relatively small amount of dense chromatin; however, the nuclei were filled with an abundance of slightly basophilic parachromatin, in which a few particulate granules of dense chromatin were scattered. Many nuclei contained definite nucleoli, and many showed fine linen-like fibrils transversing their substance. A relatively narrow zone of faintly acidophilic cytoplasm surrounded each nucleus (hematoxylin and eosin stain), no definite cell wall was present, and the cells, although they were apparently individually separated and independent (not arranged as a syncytium), were not sharply outlined. A considerable number of the cells were in stages of mitotic division. Frozen sections stained with sudan III and nile blue sulfate demonstrated no neutral fat or lipoid in these cells.

In most parts of the spleen the malpighian corpuscles had disappeared and those that remained were very small and partially disorganized (fig. 2 *B*). There was not complete disorganization of the splenic architecture, however, although the pulp cords and sinusoids were partially obscured by the crowding in them of cells derived from the reticulo-endothelium (figs. 1 *B* and 2 *A*). The trabeculae were more widely separated than in the normal organ. A considerable amount of brown pigment was scattered through the spleen in the form of dense masses or fine particles (figs. 1 *B* and 2 *A*); this was apparently not "formaldehyde pigment," for it was not found in any of the other organs.

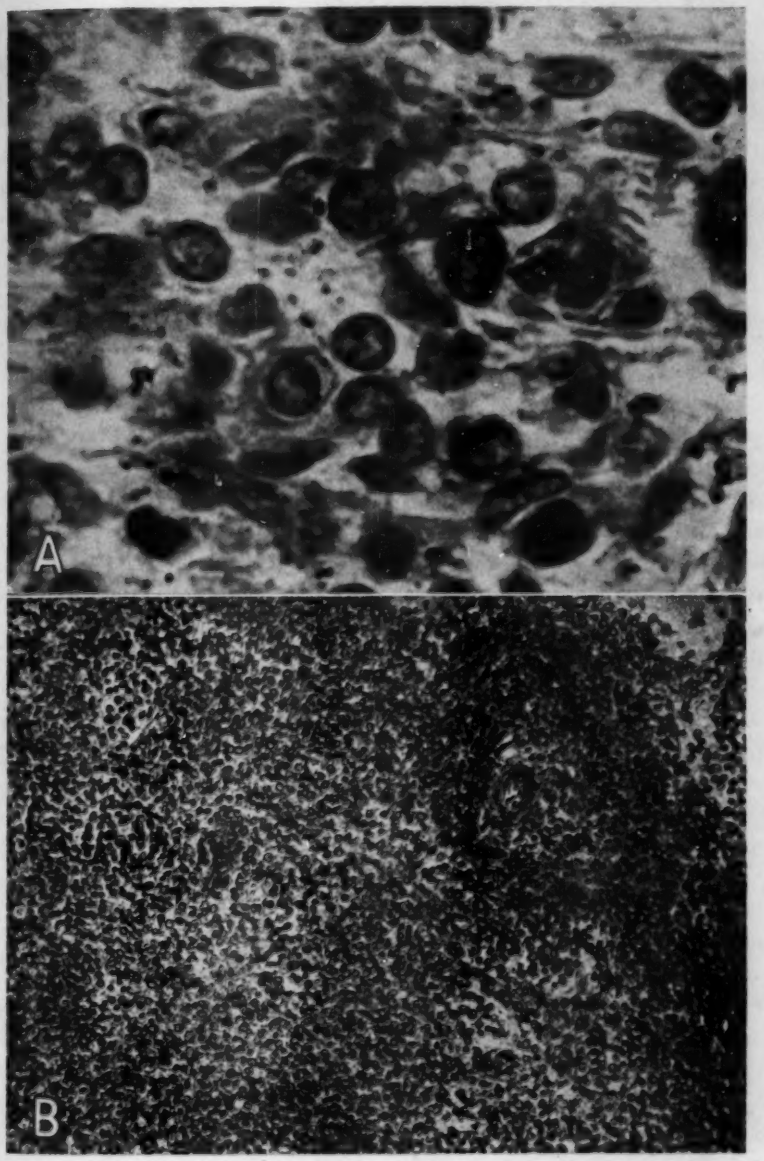

Fig. 2.—*A*, high power photomicrograph of the spleen to show the cytologic character of the reticulo-endothelial cells. The dark specks are pigment granules. *B*, low power photomicrograph of the spleen. Note the incomplete obliteration of the splenic structure and the persistence of a small malpighian corpuscle.

COMMENT

In general, it should be emphasized that the clinical pictures of the different forms of reticulo-endotheliosis are variable and not always clearly or sharply separable from each other on purely clinical grounds. The present case is of interest from a number of points of view. In the first place the short acute course of the disease corresponded closely with the limited extent of the anatomic lesions. Clinically the disease ran its course, with the symptoms of acute purpura (cutaneous and gastro-intestinal hemorrhages and epistaxis), in the short period of two months. Its progress was obdurate to all therapeutic measures.

Attention needs to be called to the fact that there was no history of preceding or accompanying infection. While the patient was under the closest observation in the hospital the only febrile reaction was that which temporarily followed the transfusions.

From the pathologic point of view this case apparently represents a pure type of splenic reticulo-endotheliosis. However, it is not our thesis to contend for the strictly localized nature of the disease; in fact, the converse of such a point of view is better supported by most observations. But the abbreviated clinical course, together with the limited anatomic changes, is significant and leads us to regard this case as one which terminated early in the disease and one in which the anatomic lesions may be interpreted as those of the initial period. Furthermore, the fact that involvement of the spleen is the most constant pathologic feature in all of the reported cases may ultimately bring one to think of leukemic reticulo-endotheliosis as a disease primary in this organ. In other words it may be that, whatever the etiologic factor in this disease is, it commonly appears to concentrate its earliest stimulative effect on the spleen. The leukopenia and thrombocytopenia must be regarded as evidence of an effect on the bone marrow, also, but the sections and imprints showed no anatomic alterations. It seems likely that had the disease extended through a longer time, more changes similar to those found in the spleen might have appeared in other parts of the reticulo-endothelial system. There is little wisdom in extended speculative efforts based on the study of a single case, but at a later date the findings presented here may be significantly correlated with those from other reported cases. A review of the literature is not necessary in this report, for the recent paper by Foot and Olcott [2] includes an adequate summary of previous contributions. However, the more recent papers by Giffin and Watkins,[3] Ritchie and Meyer,[4] Kato [5] and Foord, Parsons and Butt [6] should be cited.

3. Giffin, H. Z., and Watkins, C. H.: Am. J. M. Sc. **188**:761, 1934.
4. Ritchie, G., and Meyer, O. O.: Arch. Path. **22**:729, 1936.
5. Kato, K.: J. Pediat. **8**:679, 1936.
6. Foord, A. G.; Parsons, L., and Butt, E. M.: J. A. M. A. **101**:1859, 1933.

The finding of atypical reticulo-endothelial cells in the blood stream is of primary diagnostic importance, and when one observes the manner in which the splenic sinusoids are packed with these cells (figs. 1 *B* and 2) the absence of the cells from the circulating blood would certainly be more strange than that a few should be present. The recognition of these cells in blood smears is not a difficult matter for the experienced hematologist, but by many laboratory workers they are frequently misinterpreted as monocytes. The atypical reticulo-endothelial cells, when found in the blood, have a "superficial resemblance to monocytes; they are monocytoid but not true monocytes" (Downey). Whenever the possibility of this malady needs to be considered, blood smears should be repeatedly examined for monocytoid forms. Possibly the reason that so few cases of reticulo-endotheliosis have been reported in the American literature is to be found in the fact that not many pathologists have learned to identify the typical cells in blood films and to separate them from true monocytes. The extensive hyperplasia of the reticulum, with the mobilization of free monocytoid cells, produces a blood picture (fig. 1*A*) quite different from that found in ordinary myelogenous and lymphatic leukemia.

The spleen showed a uniform histologic structure so far as the cytologic picture was concerned. No unusual number of plasma cells or eosinophils was noted, and only an occasional multinucleated giant cell was found. There was nothing in the picture to argue for the inflammatory nature of this lesion. On the other hand, the rather regular crowding of the sinusoids and pulp cords with histiocytes was at variance with the wild, invasive and destructive growth of well recognized forms of malignant neoplasia. No evidence of phagocytosis by the hypertrophied cells of the splenic tumor could be made out. The retothel hyperplasia which this spleen showed was of a particular type and is best interpreted as the anatomic picture of leukemic reticulo-endotheliosis.

SUMMARY

A case of acute leukemic reticulo-endotheliosis is reported, in which the diagnosis was made during life by finding monocytoid cells in the blood. These cells had the following specific characteristics: a moderate amount of basophilic cytoplasm, in which small areas of yellowish hyaloplasm and dark azurophilic granules were present; a sievelike nucleus, the structure of which was similar to but coarser than that of a myeloblast; sometimes several large irregular indistinct nucleoli. The majority of these atypical reticulo-endothelial cells represented intermediate stages of differentiation between the reticulum and myeloblast forms; a few appeared to be differentiating toward young lymphocytes. After death hyperplasia of the reticulo-endothelium was found in the spleen only.

PRIMARY MULTILOCULAR MYCOTIC ANEURYSM OF THE AORTA

A. Reynolds Crane, M.D., Boston

In using the term "primary mycotic aneurysm," I refer to a lesion developing in the wall of an artery which is not associated with any demonstrable intravascular inflammatory focus, as bacterial endocarditis, or with any inflammatory process in the surrounding tissues. In the vast majority of cases an aneurysm due to destruction of the wall of an artery by bacteria is associated with bacterial endocarditis and is therefore secondary, developing in the aorta by direct extension from vegetations on the aortic valve, as maintained by Grant,[1] or resulting from lodgment of bacterial emboli from the valves in the vasa vasorum, as suggested by Osler.[2] In the series of 217 cases of mycotic aneurysms assembled by Stengel and Wolferth,[3] including their own and instances from the literature, the aneurysm was found associated with valvular disease in 187 cases.

Mycotic aneurysm is well recognized but is of infrequent occurrence. Garland [4] reported four instances in 167 cases of aneurysm, or 2.4 per cent. Five instances of myoctic aneurysms have been found at autopsies since 1912 at the Boston City Hospital in 152 cases of aneurysm (this does not include cases of diffuse dilatation of a lesser degree without actual formation of an aneurysm). Mycotic aneurysm of the aorta itself is still rarer, Stengel and Wolferth reporting 66 instances in their series of 217 cases. The aorta, however, was the most frequently involved blood vessel, and the superior mesenteric artery and its branches were the next most frequently involved. In Garland's series there were only 2 cases of mycotic aneurysm of the aorta among 94 cases of aortic aneurysm. In both of these cases the aneurysm occurred in the sinuses of Valsalva. Brindley and Schwab [5] reported 2 cases of mycotic aneurysm in a series of 100 cases of aortic aneurysm. In the group observed in the Boston City Hospital, there were 3 cases of mycotic aneurysm of the aorta among 135 cases of aortic aneurysm. In 2 of these the aneurysm involved the sinuses of Valsalva seccondary to bacterial growths on the aortic valve cusps. In the third case the aneurysm involved the ascending portion of the aorta, and this is the case to be reported here. Mycotic aneurysm of the aorta in the

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From the Mallory Institute of Pathology, Boston City Hospital.

1. Grant, R. I.: Heart **11**:9, 1924.

2. Osler, W.: The Principles and Practice of Medicine, ed. 11, revised by T. McCrae, New York, D. Appleton and Company, 1931, p. 868.

3. Stengel, A., and Wolferth, C. C.: Arch. Int. Med. **31**:527, 1923.

4. Garland, H. G.: J. Path. & Bact. **35**:333, 1932.

5. Brindley, P., and Schwab, E. H.: Texas State J. Med. **25**:757, 1930.

absence of valvular disease is very unusual,[6] and in this respect the following case is of interest.

REPORT OF CASE

A 35 year old white married man was admitted to the Cambridge City Hospital June 30, 1936, complaining of pain and swelling of the right foot of five days' duration. There was no history of injury. The pain had come on gradually and had been gradually increasing in severity. The family history was not significant. He gave a history of rheumatic fever associated with chorea and of scarlet fever in childhood. He also had had lobar pneumonia many years ago. The remainder of the history was entirely irrelative. He stated that he had never had syphilis. There were no cardiorespiratory symptoms, and he had been entirely well up to the time of his admission.

The heart was found within normal limits by percussion and offered only a faint apical systolic murmur. The right foot showed redness and swelling over the dorsum without any evidence of laceration or abrasion. There were no evidences of lymphangitis or phlebitis. One small lymph node, not tender, was palpable in the right groin. On admission there was leukocytosis of 20,600 with about 82 per cent polymorphonuclear leukocytes. The hemoglobin was 80 per cent, and the red blood cells, 4,500,000. The results of urinalysis, the Hinton test and blood culture were negative.

The foot was treated with warm wet soaks, and the swelling and redness disappeared within a week, although the patient continued to complain of pain in the foot until his death on Jan. 1, 1937. Throughout his stay his temperature indicated sepsis, "spiking" almost daily to from 102 to 104 F. The white blood cell count varied from 15,000 to 24,000, with essentially the same differential percentages as on admission, until one week before death, when the count rose to 36,000. The hemoglobin and red blood cells fell steadily to 45 per cent and 2,400,000, respectively, despite repeated transfusions. Repeated blood cultures throughout the course were negative. Sputum disclosed no tubercle bacilli; a prostatic smear revealed neither pus nor organisms. The Widal reaction was negative. X-ray pictures of the sinuses, chest and right foot presented nothing of importance. An x-ray picture of the heart taken (at a distance of 7 feet [2 meters]) late in the course showed no increase in any of the diameters.

Sept. 3, 1936, about two months after admission, a loud systolic murmur was heard over the aortic area for the first time. This was transmitted to the great vessels of the neck. Also present over the aortic area was a soft diastolic murmur. These murmurs persisted throughout the remainder of the course. September 20 he had a sudden knifelike pain in the left upper quadrant of the abdomen, associated with marked tenderness over the spleen, which, however, could not be palpated. The urine remained normal, and the sequence was interpreted as indicating a splenic infarct. One month later he had a similar sudden sharp pain in the left costovertebral angle. At this time the urine showed many red cells, and the episode was regarded as evidence of a left renal infarct. At no time during this period were any petechiae noted, and repeated blood cultures remained negative. He became slowly but progressively worse, losing much weight and showing marked secondary anemia. About one week before death it was noted that spots appeared on the dependent portions of his body when he main-

6. Clerc, A., and Bascourret, M.: Bull. et mém. Soc. méd. d. hôp. de Paris 53:285, 1929. Grant.[1] Stengel and Wolferth.[8]

tained one position for any period. About this time numerous râles began to be heard in his chest. He became comatose, and petechiae appeared in the conjunctiva and soft palate on the day of his death.

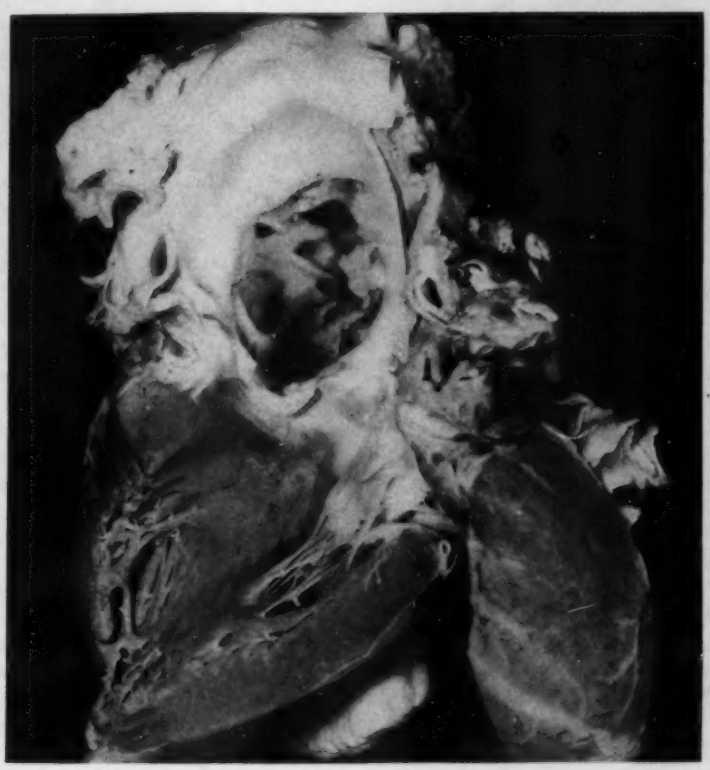
The clinical diagnosis was: rheumatic heart disease with mitral stenosis; subacute bacterial endocarditis of the aortic valve.

Postmortem Observations.—Autopsy was performed two and one half hours after death. The body was that of a well developed but poorly nourished and extremely anemic white man. There were numerous petechiae of the conjunctivae, torso and extremities and numerous irregular purpuric lesions over the torso and extremities. The right foot showed no evidence of inflammation. The peritoneal cavity contained about 500 cc. of clear amber fluid but was otherwise normal. The left pleural cavity contained about 500 cc. and the right about 1,000 cc. of similar fluid. There were recent diffuse fibrinous adhesions over the upper lobe of the right lung and old fibrous adhesions binding the lower lobe of this lung to the diaphragm. No thymic tissue could be identified. The pericardial cavity was normal. The heart weighed about 370 Gm. The pericardium and the right side of the heart were entirely without abnormality, as were the left auricle, ventricle and coronary arteries. The mitral valve measured 8 cm. in circumference and showed a slight white rubbery thickening of the cusps and chordae tendineae, but without stenosis. Along the line of closure, on the auricular surface, was a row of very fine sandpaper-like granules, all less than 0.4 mm. in diameter. The aortic valve measured 6 cm. and also showed mild diffuse white rubbery thickening of the cusps with fusion at the commissures for a distance of from 0.2 to 0.3 cm. In addition, at the bases of the cusps there were several calcific nodules projecting into the sinuses of Valsalva. There were no vegetations. The left lung weighed 440 Gm. and was essentially normal save for a narrow rim of marginal atelectasis in the lower lobe. The right lung weighed 1,350 Gm. and showed typical pneumonic consolidation of the entire upper lobe. The spleen weighed 390 Gm. and showed several typical wedge-shaped anemic infarcts from 3 to 5 cm. in diameter. The liver weighed 1,550 Gm. and was normal. The combined weight of the kidneys was 390 Gm., and each showed several typical anemic infarcts, from 2 to 4 cm. in diameter, involving the cortex and medulla. In addition there were numerous petechiae on both the capsular and cut surfaces. The remaining abdominal and pelvic organs were normal.

The acending portion of the aorta showed an aneurysm of the right anterior wall. This aneurysm began at the sinuses of Valsalva and extended up to the transverse portion of the arch to measure about 6 cm. in diameter. The aneurysm was irregularly divided into small pouches, from 1 to 2 cm. in diameter, most of which seemed to have a smooth white lining similar to that of the rest of the aorta. At three points these pouches communicated directly with a second aneurysmal sac, measuring about 6 by 4 by 3 cm., with a wall 0.6 cm. thick, which seemed to lie between the media and the adventitia. This second sac extended posteriorly toward the base of the heart from the junction of the ascending and transverse portions of the thoracic aorta. It had an irregular grayish white lining and contained only fluid blood. At the margins of the communications between the aneurysm proper and the outer sac were large white vegetations, soft and friable, varying from 0.5 to 1 cm. in thickness and from 1.5 to 2 cm. in length. At one point on the posterior wall of the aorta, at the left, about 3 cm. from the aortic valve, uninvolved in the aneurysm, was a flat area of sandpaper-like granules, 1 by 1.5 by 0.1 cm., similar to those noted on the mitral valve. The remainder of the aorta was normal but of the hypoplastic type, measuring only 4.5 cm. in circum-

ference and appearing translucent when held up to the light. In addition to the aortic aneurysm there was an aneurysmal dilatation of the superior mesenteric artery, 5 cm. in diameter, between the middle colic and ileocolic branches.

The anatomic diagnosis was: lobar pneumonia involving the upper lobe of the right lung; mycotic aneurysm of the arch of the aorta with vegetative aortitis; mycotic aneurysm of the superior mesenteric artery; congenital hypoplasia of the aorta; rheumatic heart disease with involvement of the mitral and aortic valves; bilateral hydrothorax; ascites; diffuse fibrous pleuritis in the right side of the chest; anemic infarcts of the spleen and kidneys; focal embolic glomerulonephritis.



Reproduction of specimen, about one-half actual size, showing the multilocular appearance of the aortic aneurysm. The vegetations may be seen extending into the lumen on the right side.

Bacteriologically, a pneumococcus (type XII) was obtained from the upper lobe of the right lung, and cultures of the cardiac blood and aortic vegetations yielded no growth.

Histologic Observations.—The sections from the upper lobe of the right lung showed a picture typical of lobar pneumonia, the alveoli being distended with serous exudate, fibrin, polymorphonuclear leukocytes and macrophages. Sections of the splenic and renal infarcts showed typical anemic infarction with central coagulation necrosis and a margin of polymorphonuclear leukocytes, red cells and macro-

phages. In the splenic infarcts several small clusters of gram-positive cocci in pairs and short chains could be identified. The kidneys also showed typical focal embolic glomerulonephritis with epithelial crescents in the glomerular spaces and hemorrhage into the tubules. The adrenals revealed only a marked decrease in the lipoid content of the cortical cells. The remaining abdominal and pelvic organs were histologically normal. There was marked hyperplasia of the vertebral marrow but with normal maturation in both the red and the white series.

Of particular interest were the sections from the aneurysms and from the heart. The myocardium showed several old, fairly large perivascular fibrous scars with fibrous tissue strands extending between some of the neighborhing muscle fibers. In addition there were scattered small areas of more recent necrosis from which the muscle fibers were absent and which showed scant infiltration by a few lymphocytes, macrophages and eosinophils. There was no necrosis of collagen nor were there any lesions suggestive of Aschoff bodies. Sections of the mitral valve disclosed a slight increase in collagen without any inflammatory reaction. The granules on the mitral valve noted grossly were composed of fibrin and a few scattered gram-positive cocci in pairs and short chains. There was no infiltration or reaction in the underlying tissue, and the picture was more that of a marantic thrombus than that of an active process. Sections of the aortic valve again showed collagenous thickening and some vascularization at the base, but without any inflammatory reaction. There was old calcification at the base but no evidence of bacteria at any point.

Numerous sections of the aneurysm were taken. The sections of the outer wall of the outer aneurysmal sac showed a dense outer rim of collagen containing a few fragmented elastic fibers and an inner margin of old fibrin and necrotic polymorphonuclear leukocytes, which portion was undergoing organization with active proliferation of fibroblasts. The sections of the inner wall of this outer sac disclosed a similar picture, but the underlying aorta was normal. There was, however, collagenous thickening of the adventitia with lymphocytic and plasma cell infiltration about the vasa vasorum. This infiltration was not present in the media, and there was no medial necrosis that would suggest either a syphilitic or a rheumatic aortitis. There were numerous macrophages laden with a golden brown iron-containing pigment in the adventitia.

The sections taken through the locules in the aortic aneurysm itself presented a very similar picture, with a thin wall of dense collagen containing only scattered small fragments of elastic tissue and an inner margin similar to that of the outer aneurysmal sac. There was no endothelial lining although the surfaces appeared smooth grossly. Sections through the granules on the aorta showed old fibrin undergoing organization lying on the surface of a normal aortic wall. These granules contained no organisms. However, outside of the aorta at this point was the dependent portion of the outer sac, which here showed a much more acute process with numerous macrophages, polymorphonuclear leukocytes and fibrin. The sections taken through the opening between the aneurysm proper and the outer sac and including the vegetations showed the normal aortic wall to stop abruptly. There were loss and fragmentation of the elastica about the openings and a marked acute inflammatory reaction with fibrin, polymorphonuclear leukocytes and macrophages. The vegetations were composed, for the most part, of old, partially organized fibrin but also contained numerous gram-positive cocci in pairs and short chains. No organisms could be identified definitely in any other portion of the aneurysm.

The sections of the mesenteric aneurysm had a narrow outer hyaline rim under the adventitia, containing no elastica. There was an inner margin of old fibrin undergoing organization and containing many necrotic leukocytes. The picture was essentially the same as that of the outer aneurysmal sac of the aorta. No organisms were seen.

The sections of the aorta taken at points removed from the aneurysm and even those close to it were entirely free from pathologic changes.

These sections were stained with hematoxylin and eosin, Verhoeff's elastic tissue stain, counterstained with Van Gieson's connective tissue stain, and the MacCallum-Goodpasture stain for micro-organisms.

COMMENT

Stengel and Wolferth [3] listed the following causes of nonsyphilitic aortic aneurysms: bacterial infections, trauma, arteriosclerosis, congenital defects, traction aneurysms, vascular tumors and chemical erosions. In the case presented here, all of these are ruled out with the exception of bacterial infections and congenital defects, each of which probably played an important rôle. They further described two main modes of development in aneurysms of bacterial origin. The first is by invasion of the blood vessel wall from without, for which there is no evidence in this case. The second is by invasion of the blood vessel wall from within which may be (1) by a septic embolus landing in a small vessel or in one of the vasa vasorum, (2) by settling of bacteria on the surface of a vessel and (3) by direct extension from a valvular lesion. The third mode of origin was impossible in this case because of the absence of any active valvular lesion. The small marantic type of growth on the mitral valve is of no significance and must have developed terminally. The first mode is likewise unlikely because of the absence of any source for embolic phenomena. Thus by a process of exclusion one has the two factors leading to formation of an aneurysm in this instance. The first factor is the congenital hypoplasia of the aorta, which vessel was of the type seen in status thymicolymphaticus. An aorta that is thin, elastic and smooth, with a circumference of only 4.5 cm., is distinctly abnormal for a normally developed man of 35 years. The second factor is the presence of organisms in the circulating blood, which must have been there, from the clinical observations, despite the repeatedly negative cultures.

Why the aneurysm should have developed at the site it did is difficult to say. The aneurysmal sac and particularly the second outer sac were located in the spiral line of maximum impact of the aortic blood stream as described by von Rindfleisch.[7] The principal points in this line are: (1) the ventral aspect of the aortic bulb, (2) the middle of the ventral aspect of the ascending portion of the aorta, (3) the junction of the ascending and transverse portions of the aorta, (4) the dorsal aspect of the transverse arch, (5) the left lateral aspect of the descending portion of the aorta and (6) the dorsal aspect of the descending portion of the aorta. The second and third are the important points in regard to this case. The fact that congenital anomalies of the vascular system

7. Rindfleisch, G. E.: Lehrbuch der pathologische Gewebelehre, ed. 6, Leipzig, W. Engelmann, 1886, p. 227.

predispose to bacterial invasion is well known.[8] I believe the combination of poor development of the aorta with the force of the blood stream allowed the lodging of bacteria at the point where the anneurysm developed in this case. The size and diffuseness of the process and the presence of an outer rim of fibrosed and hyalinized aortic wall about all portions of the aneurysm support this contention.

Although the causative organism could not be identified bacteriologically, the occurrence of gram-positive cocci in pairs and short chains (probably streptococci) in the vegetations, the presence of focal embolic glomerulonephritis and the purulent character of portions of the aneurysmal sacs all point to a pyogenic organism as the direct cause of the aortic and mesenteric lesions. There were no clinical, serologic, gross or microscopic observations that in any way suggest a syphilitic process. The definite rheumatic history immediately calls to mind the possibility of a rheumatic aneurysm, as has been so frequently suggested by the French school.[9] This possibility was carefully considered, and no lesions resembling those described by Klotz,[10] Pappenheimer and Von Glahn[11] and others[12] could be found. The cellular infiltrations about the aneurysm were all present in the adventitia and composed entirely of lymphocytes, plasma cells and macrophages. There was no necrosis of collagen, Aschoff nodules or cellular infiltration as described by those investigators. The perivascular scars present in the myocardium are regarded as evidences of an old rheumatic infection but the more recent lesions were not rheumatic but of the type seen with toxemia. They most closely resembled the myocardial lesions of diphtheria and those seen in streptococcic infections. The aortic granules must be regarded as marantic thrombi in the absence of any organisms or inflammatory reaction.

The presence of an aneurysm of the superior mesenteric artery is in keeping with the known multiplicity of mycotic aneurysms. Next to the aorta it is the most frequently involved blood vessel. In this case the appearance of the superior mesenteric aneurysm is not essentially different from the aortic, although it is tempting to consider the former secondary to the latter.

It seems that in this man cellulitis of the right foot developed from which organisms invaded the blood stream to establish an intravascular septic focus in the aortic wall, producing septicemia. While the man was under observation and about two months after the appearance of the initial lesion, a mycotic aneurysm of the aorta became evident. This

8. Boyd, W.: A Textbook of Pathology, Philadelphia, Lea & Febiger, 1932, p. 341. Stengel and Wolferth.[3]

9. (*a*) Rist, E., and Vérau, P.: Ann. de méd. **34**:341, 1933. (*b*) Pappenheimer, A. M., and von Glahn, W. G.: J. M. Research **44**:489, 1924.

10. Klotz, O.: Tr. A. Am. Physicians **27**:181, 1912.

11. Pappenheimer and Von Glahn.[9b] Von Glahn, W. C.: Am. J. Path. **2**:1, 1926. Von Glahn, W. C., and Pappenheimer, A. M.: ibid. **2**:235, 1926. Pappenheimer, A. M., and Von Glahn, W. C.: ibid. **3**:583, 1927.

12. Perla, D., and Deutsch, M.: Am. J. Path. **5**:45, 1929. Gray, S. H., and Aitken, L.: Arch. Path. **8**:451, 1929. Kugel, M. A., and Epstein, E. Z.: ibid. **6**:247, 1928. Leary, T. L.: ibid. **13**:1, 1932.

was responsible for the murmurs noted clinically. Bacterial vegetations persisted at the margins of the aneurysm from which developed embolic lesions in the spleen and kidney and a focus of embolic glomerulonephritis. He finally contracted lobar pneumonia, which precipitated his death.

SUMMARY

The incidence and etiology of mycotic aneurysm are briefly discussed with special reference to mycotic aneurysm of the aorta. The term "primary mycotic aneurysm" is suggested for mycotic aneurysm which is not associated with any intravascular septic focus, as bacterial endocarditis, or with any neighboring inflammatory process.

A case of primary mycotic aneurysm of the aorta composed of multiple locules and associated with congenital hypoplasia of the aorta and mycotic aneurysm of the superior mesenteric artery is reported.

General Review

SHOCK, ITS MECHANISM AND PATHOLOGY

VIRGIL H. MOON, M.D.
PHILADELPHIA

HISTORICAL REVIEW

Early medical writings[1] contain occasional descriptions of shock-like phenomena under circumstances in which no vital structures had been injured. Latta (1795) is said to have been the first to apply the term "shock" to such an occurrence. Early observers applied the term loosely to diverse conditions associated with sudden weakness, fainting, unconsciousness and sudden death. Distinctions were not drawn between mechanical impacts, such as the shock of a fall or the concussion of a blow to the head, fright or grief, as the shock of a horrible sight or of sad news, and the grave collapse, or "wound torpor," which develops following extensive trauma or burns. These and like conditions were discussed indiscriminately as shock.

The earlier hypotheses were entirely conjectural. Foreign substances in wounds and coagulation and other changes in the blood were proposed as causes. The writings abound in such hypothetic concepts as "draining of vital fluid," "loss of animal and organic powers," "destruction of the great nervous power," "complete depression of all vital functions," "commotio cerebri" and the like. A detailed review of the theories is not profitable. One of them may be cited as reflecting the character of medical thought within this period. Samuel D. Gross (1872) wrote:

> Shock is a depression of the vital powers, induced suddenly by external injury, and essentially dependent on loss of innervation. It bears the same relation to the nervous system as syncope to the vascular. In the one case the result is caused by a diminution of the nervous fluid, in the other by a diminution of the blood. In both the consequence is more or less prostration. . . . The blood has long been known by physiologists as the vital fluid so necessary to the well being of the system. But it is certainly not the only fluid entitled to this distinction; the nervous fluid is both more subtle and more important as a life preserver. When blood flows away in a mighty and overwhelming torrent . . . life is destroyed by the excessive sanguinous drainage. But in shock the same effect may happen, and yet the body be literally surcharged with blood, not a single drop, perhaps,

From the Department of Pathology, Jefferson Medical College.

1. A bibliography is appended to this article, to which occasionally specific reference is made parenthetically in the text.

having been spilled in the accident causing the fatal result. Thus of the two fluids the nervous is the more important, because the more essential to life; and its disturbance therefore a more frequent cause of death.

Gross criticized severely the practice of bleeding in shock, as contrary both to physiology and to common sense, adding the observation that when this is attempted by the ignorant "the blood generally refuses to flow, and consequently no harm is done." Evidently the decrease in the volume flow of blood in peripheral parts had impressed Gross.

Claude Bernard's demonstration of vasomotor control of the circulation was soon applied as explanatory of circulatory failure following injuries. Goltz' experiments of stopping both respiration and heart beat in frogs by striking sharp blows on the abdomen was hailed as a demonstration of shock induced by mechanical trauma which by reflex action paralyzed the heart and stopped respiration. Various hypotheses formulated on this basis indicated combinations of disturbances of the central nervous system with reflex inhibitions of the action of the heart, of respiration and of vascular tonus. At that time it was not realized that neither the heart's action nor respiration is paralyzed or inhibited in shock.

Those interested in these hypotheses will find them summarized critically in a monograph by Groeningen (1885). His conclusions are tersely and somewhat dogmatically stated. Some of them are not harmonious with subsequent developments, but his analysis of the evidence then available makes this the outstanding contribution of the period. The clinical features, including weakness, thirst, vomiting, rapid irregular pulse, declining temperature and blood pressure, decreased motility, sensibility and sensitivity to stimuli, lowered activity of the reflexes and general functional depression, were clearly stated. The similarity of these signs to those of hemorrhage was recognized. Failure to differentiate between shock and sudden death from other causes was cited as a source of confusion and of statistical inaccuracies. Fat embolism, coagulation and other changes in the blood were rejected as causes. Use of poor instruments, unskilful manipulation, fatigue and deprivation, lowering of resistance as a result of hemorrhage, disease, alcoholism or undernourishment, anxiety, restlessness and fear on the part of the patient, improper bandaging, uncareful handling and rough transportation of the wounded were stressed as contributing factors. The presence of any of these factors justified delay in operation, and the use of stimulants and rest in a warm bed were advised in the interim. He stated that a few hours usually elapsed between the injury or operation and the development of shock, but that if the latter did not develop within twenty-four hours it seldom occurred later. These observations received remarkable confirmation during the World War thirty years later.

HYPOTHESIS OF "EXHAUSTION"

Groeningen found that previous hypotheses were contrary to the physiologic evidence and sought for a satisfactory explanation in dysfunction of the vasomotor mechanism. He reasoned that any traumatic insult produces exhaustion of nerve centers in a degree proportional to the severity of the injury. Every stimulation to a nerve is fatiguing to it and to the central nervous system. Repeated or prolonged stimulation results in decreased reactions. The summation of the stimulations arising from preceding conditions, from the injury or operation itself and from subsequent events results in exhaustion of the vital centers, particularly those in the medulla. All the manifestations formerly attributed to reflex inhibitions and reflex paralyses were thus explained to his satisfaction.

Crile seems to have adopted this hypothesis *in toto*. He produced shock in dogs by various combinations of trauma, stimulation and evulsion of nerves, manipulation of the viscera and other forms of tissue abuse. The effects of these were shown not to be of cardiac origin. The immediate result of such injuries was an increase in blood pressure, indicating vasomotor responsiveness and activity. But after prolonged repeated injuries the pressure declined and the circulation became ineffective, suggesting vasomotor inactivity attributable to exhaustion.

He believed that repeated excessive painful stimulation exhausted the vasomotor centers in the cord and medulla, as a result of which the reflexes became weakened until finally arterial relaxation occurred, that these effects were produced even under complete anesthesia, when no consciousness of pain was present, and that the decline in blood pressure was due to arterial relaxation incident to overstimulation. A corresponding fall in venous pressure was shown, and the defect in the circulation was attributed to an insufficient return of venous blood to the heart.

A few postmortem observations were published. Venous congestion of the viscera was described, but in no instance was dilatation of the arteries observed. This discrepancy in the evidence apparently was overlooked. A microscopic search for evidence of exhaustion in the cells of the brain revealed degenerative changes following shock. The brain cells were first hyperchromatic, later the nuclei became eccentric, there was chromatolysis, and finally there was disintegration of the cells. This was interpreted as evidence of exhaustion and degeneration resulting from excessive stimulation. Degenerative changes were present also in the parenchymatous organs. Just how these could result from excessive sensory stimulation was not shown.

Crile reported exactly similar changes in the brain and other organs following death from muscular exhaustion, starvation, insomnia, anaphylaxis, electrocution, asphyxia, prolonged anesthesia, adrenalec-

tomy, injections of epinephrine, hemorrhage, intravenous injections of acids and from other conditions. This fact materially lessens the probability that the changes are related to a nervous mechanism. In the later stages of shock there is a high degree of anoxemia. This is likewise a prominent feature in several of the other conditions mentioned. It is probable that the changes described were due to anoxemia and were the result rather than, as Crile supposed, the cause of shock.

Crile believed that the changes in shock and those in exhaustion from whatsoever cause were identical and that the tissues of the central nervous system were chiefly involved. He wished to substitute the term "exhaustion" for "shock."

Cannon states that a degree of anesthesia sufficient to abolish reflexes and to render an animal inactive is sufficient to protect the brain against afferent impulses. In other words, painful stimuli do not reach the brain under the usual surgical anesthesia.

A number of investigators (Porter; Janeway and Ewing; Seelig and Lyon; Mann; Wiggers; Phemister, and others) were unable to produce conditions resembling shock by prolonging mechanical or electrical stimulation of large numbers of sensory nerve fibers. Porter found that the vasomotor reactions in shock are not exhausted, depressed or inhibited. Mann's experiments led him to conclude that the mechanism of shock is peripheral, not central. He found evidence of increased vasomotor activity in shocked animals. Likewise Erlanger and his associates found an increase in vasomotor activity for several hours during shock. It declined only after the blood pressure had been reduced below 50 mm. and was abolished only as the animal was dying.

In terminal stages, when the blood pressure is below the critical level, the brain, as well as other organs, suffers from lack of oxygen. This affects all functions of the central nervous system. Reflexes are abolished, there is no response to painful stimuli, the animal or person is lethargic or semicomatose, the respirations become weak and shallow, and the blood pressure declines to zero. Apparently this terminal stage is the only one in which vasomotor deficiency is present in shock.

HYPOTHESIS OF ACAPNIA

Henderson found that the first evidence of shock produced in dogs by intestinal manipulation is a decrease of from 40 to 70 per cent in the volume output of arterial blood. This decrease occurs regularly before the blood pressure begins to decline. He showed that this is not due to cardiac deficiency but to diminution in the return flow of venous blood from systemic areas. He advanced the explanation that there is a venopressor center activated by carbon dioxide which controls the return of venous blood and that marked hyperpnea due to painful stimuli, anesthesia, emotional excitation and other influences

results in depletion of the carbon dioxide below physiologic limits—acapnia. The venous blood pressure then declines, the return flow of blood becomes deficient, the circulation fails, and asphyxia and shock occur. Several associated phenomena seem to support this explanation, such as lowered metabolism, anoxemia, cyanosis and congestion of the viscera, and decrease in the volume flow of blood preceding the fall in arterial blood pressure.

When subjected to the test of controlled experimentation, this attractive hypothesis received little confirmation. Mann was unable to produce shock by hyperventilation of the lungs, following the technic described by Henderson. He produced shock by inserting the hand into the abdomen through an incision. The skin was then tightly clamped about the wrist, and a constant stream of carbon dioxide was passed directly into the abdomen while the intestines were manipulated. This precaution did not prevent or retard the development of shock.

Janeway and Ewing provided a rebreathing apparatus whereby the carbon dioxide of the blood was maintained within normal limits. In other cases they supplied carbon dioxide from a tank through a respirator, whereby the carbon dioxide of the blood was kept above the normal level. Under such conditions shock was induced as readily as when no such precautions were used. From various other experiments they found no evidence that a diminution in carbon dioxide is a causative factor in shock. Likewise Wiggers was unable to confirm the hypothesis of acapnia. Coonse and his collaborators found that when an animal in shock was given inhalations of carbon dioxide its condition became rapidly worse.

Cannon showed that the assumption of increased respiratory activity following wounds is invalid. The unanimous experience of army surgeons is that the wounded feel no pain at the moment of being struck. The impact of a projectile seems to deaden the adjacent nerves, and pain is a feature only when inflammation has developed. Also, there is no hyperpnea following severe wounds. Typically the breathnig seen in the casualty clearing stations, while somewhat faster than normal, is superficial and produces less effective ventilation of the lungs than normal breathing would. Also, conditions of intolerable pain, such as the passage of biliary or renal calculi or facial neuralgia, do not result in shocklike phenomena. He concluded that "pain and the excessive breathing required for acapnia are commonly absent. Shock may exist without acapnia, and acapnia may exist without shock. The low CO_2 content of the blood in shock may be accounted for as the result of the low blood pressure, not as its cause."

Further evidence against the validity of the hypothesis that shock is due to acapnia may be found in the production of shock by means which cause no variation in the respiratory rate. Shock resulting from

injections of histamine, peptone, tissue extracts or bile or from the introduction of muscle substance or of extracts of muscle into the peritoneal cavity or from anaphylaxis cannot be explained satisfactorily on the basis of a lowering of the carbon dioxide content of the blood or of the tissues.

THEORY OF FAT EMBOLISM

Groeningen discussed the occurrence of a condition resembling shock following extensive injuries, especially those in which long bones have been fractured or comminuted. After such injuries, also following amputation, patients who have been apparently in good condition for perhaps twenty hours may manifest restlessness and anxiety, air hunger, rapid respiration, pallor, falling temperature, falling blood pressure, rapid pulse, which becomes impalpable as the condition progresses, vomiting of blood-tinged fluid and acute pulmonary edema. He ascribed these phenomena to the plugging of the pulmonary capillaries with fat taken up from the areas of injury and carried by the blood stream to the lungs. He maintained that only an unskilled person would mistake this condition for shock. However, those who have seen shock develop after extensive injuries or burns will note that it presents features identical with those described by Groeningen and will feel that his differentiation is open to question.

Warthin in 1913 published a comprehensive review of traumatic lipemia and fat embolism. Many of his observations would apply equally well to shock. Among the conditions of the occurrence of traumatic lipemia and fat embolism were listed: All varieties of injury to bone, especially comminuted fractures, extensive surgical procedures, severe physical injuries, acute peritonitis and burns of the skin. The following symptoms mentioned resemble those of shock: restlessness, vomiting, falling or rising temperature, dyspnea, signs of pulmonary edema, blood-tinged frothy sputum and collapse. Several of the pathologic features which he described are indistinguishable from those found in shock from causes which excluded fat embolism. Some of these are pulmonary congestion, edema, capillary hemorrhages, a tendency toward bronchopneumonia, marked congestion of the viscera and miliary hemorrhages in the heart, brain, kidneys and elsewhere. He emphasized the finding of free fat in the sputum, in the blood and in the capillaries of various organs as distinctive features, essential to the diagnosis. One has the feeling that in the absence of these features many deaths attributed to fat embolism, including a few reported by Warthin and by others whom he quotes, were probably due to shock.

Porter found that olive oil or thick cream injected intravenously into cats produced a marked fall in blood pressure and other manifestations like those of traumatic shock in man. Bissel injected into dogs

intravenously varying amounts of olive oil. Small quantities were without effect. Larger and repeated injections caused finally a sudden rise in venous pressure and a gradual fall in arterial pressure, followed by death. This effect was confirmed by Simonds, but the amount of olive oil necessary was about 2 cc. per kilogram of body weight. Later Porter produced a fall in blood pressure by injecting fats into the vertebral arteries and believed that the circulatory phenomenon of fat embolism resulted from the plugging of capillaries in the medulla by particles of fat.

In evaluating the results just described, it must be emphasized that shock is always accompanied by a fall in venous pressure. Many observers have noted that this precedes the decline of arterial pressure. Wiggers studied the effects of injecting fats into the circulation, both arterial and venous, and found them extremely variable. Circulatory disturbances comparable to those of shock produced by manipulation of the intestines did not result.

Crile found the theory of fat embolism inadequate. He noted that shock resulted from abdominal injuries which penetrated the viscera but not from similar injuries in which no penetration occurred, though the same areas of fatty tissue had suffered similar trauma. He called attention to the occurrence of shock following burns and following injuries to the head and chest, conditions in which no fatty tissue had suffered.

Cannon found that the theory of fat embolism lacked evidence to support it. With plugging of pulmonary capillaries, venous pressure should be increased and the systemic veins should be distended. In shock venous pressure is decreased, and the systemic veins are collapsed and relatively bloodless. The production of shock by manipulation of the intestines, by injections of peptone, fat-free tissue extracts or histamine or by anaphylaxis leads one to conclude that the entrance of fat into the blood stream is not essential to the production of shock. This conclusion does not contradict the fact that under some conditions fat may enter the blood stream in quantities sufficient to affect the circulation seriously or even to cause death. Such occurrences are rare and should be differentiated from traumatic shock. In reviewing reports on fat embolism one is impressed by the probability that many of the conditions described were in fact traumatic shock and that they were attributed to fat embolism on insufficient evidence.

Moon and his associates (Moon, references f, i, j and k) produced shock by various means which furnished no opportunity for the entrance of fat into the circulation. They repeatedly examined frozen sections without finding stainable fat within capillaries. Others have reported similar results. The evidence indicates that fat embolism affords no adequate explanation of the mechanism of shock.

HYPOTHESIS OF DECREASE IN THE ALKALI RESERVE

Henderson observed that deficient oxidation is prominent in experimental shock and suggested that this produces increased acidity in the tissues and blood. Crile included shock among the clinical conditions in which acidosis is an important factor. Cannon (reference c) found a marked decrease in the alkali reserve in cases of shock, in cases of severe hemorrhage and in cases of gas bacillus infection. This state paralleled fairly closely the decline in blood pressure in each of these conditions. Also in a series of thirty cases of traumatic shock the gradual decline in the alkali reserve was accompanied by a progressive increase in the respiratory rate. Air hunger was manifested when the acidosis was extreme.

In a subject in which disagreement and controversy hold sway it is refreshing to find a point on which opinion is unanimous. There appears to be no dispute concerning a diminution of the alkali reserve in traumatic shock. The question at issue is not whether such a decrease occurs but how it is to be interpreted.

McElroy found a gradual decrease in the reserve alkalinity as shock developed In no case did the decrease precede the onset of shock nor was the decrease in alkalinity sufficient to account for the condition of the animal. In experimental acidosis produced by intravenous injection of lactic acid, the alkali reserve was lowered to the degree found in shock without producing marked symptoms. When by further injections the state of the animals had been reduced to a terminal one, treatment by injection of sodium bicarbonate solution resulted in prompt recovery. Similar treatment of animals in shock produced no benefit. The maintenance of the alkali reserve at normal levels by injections of sodium bicarbonate solution did not prevent the development of shock. He concluded that acidosis is not a cause but is a secondary associated condition. Similar experiments and results were reported by others (Guthrie; McLeod; the Special Committee on Surgical Shock and Allied Conditions of the Medical Research Committee, and other investigators). The conclusion was drawn that acidosis, or a reduction in the reserve alkalinity of the blood, is not the cause of shock or even an important factor in its production.

Peters and Van Slyke supported the observation that alkalosis is replaced by acidosis in conditions of anoxia. They explained this as due to the defective oxidation of carbohydrates, which results in the formation of lactic acid and perhaps of other acid metabolites. They found that this may occur in asphyxia, shock, hemorrhage or anesthesia induced with ether or chloroform.

Apparently the decrease in the reserve alkalinity of the blood in shock is the result of decrease in oxidation due to deficiency of the circulation. It is the effect and not the cause of the disturbance in the circulation. It develops similarly whenever anoxia is marked.

THEORY OF TRAUMATIC TOXEMIA

During the World War shock following wounds incurred in battle became a major problem for the medical service of the allied forces. The British Medical Research Committee organized an investigation on its nature and management. The Special Committee on Surgical Shock and Allied Conditions consisted of men of recognized ability and wide experience in their respective fields. Various phases of the problem were investigated by men whose qualifications were adapted to these particular phases. No one attempted the solution of the problem as a whole. The casualties of battle provided a wealth of material, which has never been equaled. The plague of war furnished opportunities for clinical studies on shock such as a great pandemic disease would provide for investigations concerning its nature. Field observations were checked and compared with experimental studies. The task of summarizing and digesting the individual reports was undertaken by Cannon. His monograph on traumatic shock combined the findings from these investigations with evidence from other sources.

It was found that shock resulted most frequently from certain types of wounds. These included multiple wounds from shell fragments which collectively had caused much laceration of tissues; compound or comminuted fractures associated with extensive laceration, and extensive deep injuries to soft tissues. The impact of projectiles traveling at high velocity was transmitted laterally and produced wide areas of destruction in the tissues surrounding the path of the projectile. Such wounds were not accompanied by much hemorrhage. Circulatory deficiency did not develop immediately but usually made its appearance from four to twenty-four hours after the injury. Shock resulted much earlier from injuries involving the abdominal viscera.

Everything favoring absorption from the injured area favored the development of shock. It developed most readily when the area of damage communicated with the surface by only a small opening. Conversely when a large area of flesh and skin had been completely carried away, shock was either slight or entirely absent. The time elapsing before surgical treatment of the wounds was an important factor. In a large series of similar wounds the mortality was only 11 per cent when the subjects were operated on within three hours. The mortality rose to 37 per cent when operation was delayed for from three to six hours, and to 75 per cent following delays of from six to nine hours. This progressive increase in mortality was not attributed to infection for, although all battle wounds were infected, variations in its development within twelve hours were relatively unimportant.

It was noted that hunger, fatigue, exposure to cold, rough transportation, poor splinting or faulty immobilization of injured parts and especially delays of several hours entailing combinations of these factors

accelerated the development of circulatory failure. Immediate surgical treatment of peripheral wounds combined with the factors mentioned often resulted unfavorably. The administration of ether frequently precipitated circulatory failure in patients whose apparent condition was not serious. Better results were obtained by postponing operation until restorative measures, stimulants and rest had made it less likely that the anesthetic and surgical procedures would precipitate shock.

It became a standard practice in the presence of extensive lacerations of a limb to perform a rapid "guillotine amputation" when the improvement following restorative measures was at its height. Sometimes a tourniquet was applied tightly above the injury to prevent absorption of substances from the damaged tissues until restorative measures had been applied. Amputation was then performed without removing the tourniquet.

Observations relative to the use of tourniquets are significant. These were often applied during first aid and were still in place when the wounded soldiers arrived at field hospitals or casualty stations. Shock frequently developed immediately after removal of the tourniquets. This happened so frequently that the use of tourniquets to control hemorrhage was discontinued. Their use to prevent the development of shock was sanctioned: "The suggestion is offered that if a limb has been so badly mangled that it cannot be saved, a tourniquet should be set close above the trauma and left in place until after amputation. The amputation should be performed proximal to the tourniquet. Thus the body is protected against toxic material which is present in the torn and smashed tissues and is likely to be absorbed" (Medical Department of the United States Army in the World War. Medical Research Committee).

Early experiences led military surgeons to abandon the conservative procedures usually employed in dealing with injuries. Such procedures were followed by high mortality from shock and from subsequent infections. The débridement of wounds received in battle was a radical procedure adopted early in the war. It resulted in fewer deaths from shock and in earlier recovery. All bruised or lacerated tissue was excised from the sides of the wound, and all blood clots and tissues infiltrated with blood were removed. This left a wounded area lined on all sides by clean viable tissue. A similar procedure was followed in dealing with small penetrating wounds.

Wallace reported marked immediate improvement in the condition of wounded soldiers following amputation of mangled limbs and following débridement. Such measures reduced both the incidence and the severity of shock. "The operation is commonly followed by a remarkable and maintained improvement so rapid and striking as to appear a direct sequel to the removal of the damaged limb" (McNee

and others). "As soon as possible there must be suppression of the trauma. This procedure is often the initial step in an extraordinary improvement in the patient's state. Experience proves that the exclusion of the focus of injury by short and radical procedures causes the symptoms of shock to disappear" (Medical Department of the U. S. Army in the World War. Medical Research Committee).

As experiences accumulated, the conviction grew that absorption of products from injured tissues was a major factor in producing the deficiency in the circulation which followed. This deficiency frequently was out of all proportion to the apparent severity of the wound and subsided in a remarkable fashion after amputation of the mangled limb or débridement of other wounds.

Blood Concentration and Blood Volume.—Cannon, Frazer and Hooper made red cell counts, hematocrit readings and hemoglobin determinations in a large series of cases of hemorrhage and of wound shock in which hemorrhage was a minor factor. After simple hemorrhage the blood showed dilution proportional to the amount of blood lost. This was confirmed by blood counts made on donors of blood before and following transfusions. Shock was characterized by a change of the opposite kind. The red cell counts ranged from 6,000,000 in mild shock to above 9,000,000 in severe shock The first noteworthy feature in severe traumatic shock was a high red cell count on capillary blood. They attributed this phenomenon to stagnation of stasis of blood in capillary areas, accompanied by transudation of the plasma into the tissue spaces, with resulting concentration of the corpuscular elements of the blood. The hemoconcentration was progressive and tended to be proportional to the degree of shock.

Bazett found red cell counts and hemoglobin estimations of great value as indicating whether shock or hemorrhage was present and in determining the condition of the patient and the operative risk. Keith confirmed these observations. He found also that a reduction of the total blood volume and of the plasma volume was the most striking and important feature in wound shock. In severe shock the plasma volume was reduced by 50 per cent. All persons in the state of shock had a marked reduction of total blood volume. In a series of such persons this ranged from 51 to 85 per cent of normal. When the blood volume was below 85 per cent, the patients showed grave symptoms; when it was below 65 per cent, their condition was critical. In one such case, a transfusion of 900 cc. of blood was given. Ninety minutes later, no hemorrhage having occurred in the meantime, the blood volume was only 150 cc. greater than that before the transfusion. In other words, 750 cc. of fluid had been withdrawn from circulation in the short space of ninety minutes.

Keith concluded that in severe shock a condition develops in which the normal processes of blood dilution fail to operate and the vascular structures are incapable of retaining colloid solutions or even whole blood. Robertson and Bock confirmed the findings of Cannon and his collaborators, of Bazett and of Keith. Wallace stated that recuperative power depended on the ability of the circulation to take up and to retain fluid. In one class of cases the circulatory system was able to absorb fluid from tissues and to retain fluid supplied therapeutically. In another class it could not absorb fluid, but the vascular structures would retain fluid if this was supplied in suitable form. In profound shock the circulatory system would neither absorb nor retain fluid, and treatment availed nothing.

Such experiences were confirmed apparently without exception, for they are set forth in the official histories of the medical services of both the British and the United States armies in the World War. These observations and the inferences drawn from them fit closely with the conception that in shock the reduction of blood volume is due to leakage of plasma through capillary walls rendered abnormally permeable to plasma and to other colloidal fluids. Such leakage of plasma causes concentration of capillary blood and, to a lesser degree, of systemic blood.

Experimental Evidence.—The interpretation that substances absorbed from injured tissue cause failure of the circulation, associated with hemoconcentration and reduction in blood volume, originated from observations on inestimable numbers of casualties in battle. Experiments were arranged to test that interpretation and to secure additional evidence. Bayliss and Cannon induced shock by traumatizing the muscles of cats. This was followed by low blood pressure, decreased blood volume, hemoconcentration and other features characteristic of shock. Interruption of all nerve paths to the brain did not affect the results. However, interruption of the return flow of fluid from the injured areas prevented development of shock, and shock occurred following release of the ligated vessels.

Bayliss noted also that withdrawal of 15 per cent of the total blood volume markedly increased the effects of trauma. Such a loss of blood was sustained without symptoms by uninjured animals. This observation illustrates the combined effects of trauma and hemorrhage. Muscle substance was extracted with hot saline solution. Injection of such extracts intravenously caused a prompt decline in blood pressure, accompanied by hemoconcentration and by increase in the volume of an intestional loop, shown plethysmographically. He recorded that the minute veins of the viscera were markedly engorged following shock.

Dale, Laidlaw and Richards produced shock by intravenous injections of histamine into anesthetized cats A dose of 1 or 2 mg. caused

an immediate fall in blood pressure, accompanied by a decrease of 40 per cent in the total blood volume. They interpreted these effects as due to leakage of plasma through abnormally permeable capillary walls and to stasis of blood in dilated capillaries. This interpretation was confirmed by examination of the viscera. The bowels were dull, dusky red, the smallest vessels were distended with dark red blood, and the tissues were moist and edematous. The arteries down to the finest arterioles were not dilated but were in a state of maximal contraction.

It was found that each of the various physiologic disturbances observed in shock following wounds received in battle were likewise present in shock produced by histamine. The reports on these experiments are often misquoted as implying the belief that histamine is the cause of shock following surgical operation or trauma. Nowhere do these authors affirm such a belief, but they recognized that the action of histamine typifies that of peptone, tissue extracts, products of protein cleavage and a group of substances which affect the circulation similarly.

Cannon's analysis of the observations on wounded men and of the experimental evidence led to the interpretation that secondary shock is due to a toxic factor absorbed from injured tissues which causes an increase in the permeability of the capillary walls and a consequent reduction of blood volume through escape of plasma into the tissues.

Many factors had been found to contribute to the development of shock following extensive wounds. Among these were hemorrhage, cold, delay in débridement or amputation, anesthesia, infection, fatigue, rough transport, loss of fluid by vomiting and perspiration, worry and other minor factors.

Corroborative Evidence.—Many products which contain protein will cause circulatory disturbances if introduced into the blood. Moderate doses cause an increased flow of lymph and this lymph has a high protein content. Such lymph originates by leakage of plasma through capillary endothelium when the permeability of the latter is greater than normal (Drinker and Field). Larger doses cause more severe disturbances, accompanied by the characteristic manifestations of shock.

Peptone was one of the lymphagogues described by Heidenhain. Its effects in increasing the flow of lymph and, if larger doses are given, causing immediate death by failure of the circulation, are well known. Hamburger found that filtrates of various bacterial growths increase the flow of lymph. Such substances cause death by failure of the circulation if given intravenously in sufficient dosage. Asher showed that bile or its salts increase the flow of lymph when given intravenously to dogs. It was shown recently (Horrall and Carlson; Harkins and Harmon and others; Moon and Morgan) that bile or its salts will produce shock to varying degrees if injected into dogs. The latter authors produced death at intervals varying from a few minutes to a few days by intravenous

injections either of bile or of sodium glycocholate. The blood of these dogs showed hemoconcentration, and the viscera at postmortem examination showed the changes characteristic of shock. Injections of foreign serums will either increase the flow of lymph or cause death by failure of the circulation, depending on the dosage, the animal used and the degree of sensitivity. The poison of actinia and of other marine animals will increase the flow of lymph and if injected in larger amount will produce death by shock (Richet). The same statement applies to snake venoms (Essex, Markowitz and Mann; Kellaway).

Turck found that products of disintegrating tissues are poisonous and believed that wound shock is due to toxic products derived from such tissues. Schäfer and Moore reported that intravenous injection of an extract of brain substance produces a marked fall in blood pressure. Vincent and Sheen reported that injection of watery extract of brain substance caused a fall in blood pressure accompanied by an increase in the volume of an intestinal loop and of the leg, as shown plethysmographically. They produced similar results with extracts of striated, nonstriated and cardiac muscle, kidney, liver, spleen, testes, ovary, pancreas and lung. They stated that these depressor effects were not produced through the agency of the vasomotor nerves but that the substances acted directly on the blood vessels. They found evidence also of a pressor substance in tissue extracts which caused a rise in blood pressure under similar experimental conditions. This effect was most marked following injection of substances extracted from nerve, muscle and kidney by cold saline solution. The depressor substance was best obtained by extracting the tissues with boiling saline solution. They suggested that boiling either inactivated the pressor substance or extracted more of the depressor substance, sufficient to mask the effects of the former. They concluded that these extracts of normal tissues affected the circulation by producing dilatation or contraction of vascular areas in the body.

Carlson, Woelfel and Powell found that filtered aqueous extracts of various organs had depressor effects when introduced into the circulation. Extracts of pancreas, salivary glands, gastro-intestinal mucosa, liver, spleen, kidney, lung, testis, thymus, thyroid and muscle were found to have this effect in varying degrees.

Popielski injected extracts of intestinal mucosa into dogs intravenously. Each of these produced a sudden marked fall in blood pressure, accompanied by acute illness, vomiting, urination, defecation of liquid stools, salivation, weakness and collapse. Similar results followed injection of extracts of brain, cord, pancreas and erythrocytes. Defibrinated blood caused similar manifestations, but blood serum did not. He attributed these phenomena to "vasodilatin," which he showed to be a constituent of normal cells. In order to obtain it, the cells must be

mechanically injured, mashed or lacerated. Uninjured cells do not yield it. These observations are of particular significance in the light of the subsequent work of Ebbecke and of Lewis. Popielski found that the heart's capacity was not impaired, that the vasomotor center was active and that the peripheral vessels were dilated. He reasoned that the effects were due to impairment of the action of the peripheral vasomotor nerves. It should be remembered that this work was done before the direct effects of various substances on the minute vessels were known. Perret reported that "myoserum," the juice pressed out of muscle, has highly toxic effects when injected.

Dale and Laidlaw in 1911-1912 found that small doses of histamine markedly increased the flow of lymph in dogs. Their later work showed that larger doses produced the typical failure of circulation seen in shock. As they saw it, the similarity between these unrelated substances lay in the fact that they have one property in common: that of injuring capillary endothelium. The central feature of the systemic effects of these agents is the decrease in blood volume which results from capillary atony, leakage of plasma and consequent hemoconcentration.

Delbet and Karajonopoulos made an autolysate of rat muscle which was free from bacteria. Intraperitoneal introduction of this into rats was followed by marked illness and death. Of twenty-two rats so treated, fourteen died within an hour and six in from four to twenty hours.

Cornioley and Kotzareff produced muscular injury in guinea-pigs and rabbits by methods similar to those used by Cannon and Bayliss. These injuries were followed by symptoms of shock, usually ending in death in a few hours. Shock was delayed or prevented by applying a tourniquet about the limb above the traumatized area or by amputation of the injured part. Extracts of the injured tissues produced evidences of shock in normal animals when injected intravenously.

Phemister and Handy found that normal erythrocytes that are traumatized by shaking produced a vasodilator substance which caused a decline in blood pressure. Similar transfusions with nontraumatized blood had no such effects. Laked blood had effects like those of traumatized red cells. They showed that histamine was not the factor causing these reactions.

Mason and his associates found that autolysis of normal liver substance produced marked intoxication, loss of blood volume and death in dogs. In these experiments a small portion of liver was clamped, excised, weighed and dropped back into the peritoneal cavity of the same animal. Examinations of the blood showed a progressive decrease in plasma volume, hemoconcentration, a delay in coagulation, a decrease in blood sugar and an increase in nonprotein nitrogen. Death followed regularly in from fifteen to twenty hours. They attributed this to toxic

substances derived by autolysis of the liver substance. Andrews and his collaborators repeated these experiments and verified Mason's results. They found also that bits of liver implanted in the chest or subcutaneously produced intoxication and death. In a later report Mason and Lemon occluded the blood supply to a portion of the liver in dogs. In these animals, low blood pressure developed and death followed in from fifteen to eighteen hours. A watery extract of this partially autolyzed liver was then made and injected into normal dogs. Small doses produced a temporary fall followed by a rise in the blood pressure. Doses of 7 or 8 cc. were usually fatal, and an injection of 10 cc. caused immediate death by shock.

Blalock's results from one group of experiments (reference d, p. 604) are similar in character to those cited. He obtained fluid from normal and from traumatized tissues of dogs in which shock had been produced by bruising the muscles. Fluid from nontraumatized extremities was injected into five dogs. In four of these there was a marked decline in the blood pressure during or immediately after the injection. One of these died five hours later. It was believed that the others would have recovered if they had been allowed to live. Fluid obtained from traumatized tissues of other dogs was injected intravenously into nine dogs. Eight of these died at periods varying from a few minutes to twenty-four hours after the injection. "In several instances autopsy revealed blood-stained fluid in the peritoneal cavity, macroscopic hemorrhages in several organs, and a thickened grayish gall bladder."

These findings are highly significant in view of Blalock's contention that shock is due to loss of blood and fluid *at the site of the trauma.* Blalock's sole criterion for shock was a decline in arterial blood pressure. By this standard these dogs died of shock produced by injecting fluids from normal and from traumatized tissues *without local loss of blood or fluid*. Such fidelity to one idea in the face of evidence incompatible with it is unusual. It is significant also that the description of the congestive changes seen post mortem in these animals is not in accord with his previous assertion that no such changes occur in shock. But those descriptions seem to coincide with the pathology of shock as described by Moon and his associates.

Moon and Kennedy produced shock in dogs by introducing finely chopped fresh dog muscle into the peritoneal cavities of normal dogs, by injecting fat-free neutralized watery extracts of muscle intraperitoneally or intravenously and by injecting similarly an autolysate of dog muscle. In later experiments watery extracts of intestinal mucosae were shown to have similar effects. In each instance there was marked hemoconcentration immediately following the procedure, with marked evidences of illness of the type usually seen in shock, and the postmortem observations were those regularly present following death by shock in

animals and in man. A detailed discussion of these observations will be given subsequently.

Coonse and his collaborators compared the effects of trauma and of hemorrhage on dogs. The blood became concentrated and decreased in volume following trauma. The opposite picture of a decreased cell count and increased volume of serum resulted after hemorrhage. They stated that the postmortem observations resembled in every particular those described by Moon and Kennedy. There was marked congestion of the liver and spleen and of the peripheral small vessels. The blood was viscous and slow to clot. The tissues after hemorrhage showed the ischemia typical of exsanguination. They attributed the circulatory disturbances to the systemic effects of substances liberated at the site of the trauma.

Capillary Physiology.—Highly important developments in knowledge concerning capillary structure and function have occurred since the World War. These have a direct bearing on the mechanism of shock. The fact that the investigators were not interested primarily in shock and that the studies were not designed to test any theory regarding it does not lessen the significance of the results.

Krogh demonstrated the enormous potential capacity of the capillary stream bed. Only a small fraction of this is open to circulation under normal conditions. If all the capillaries in the skeletal muscles were open simultaneously, their total volume capacity would about equal the normal blood volume. Other areas, as the lungs, the gastro-intestinal system and other viscera, have capillary capacities of similar magnitude. The capillaries have tonus and contractility independent of the arteries and of nerve control. Their contractility and dilatation are, partially at least, under metabolic control. Should atony and relaxation of capillary walls occur in an extensive visceral or peripheral area, the volume of blood in actual circulation would be reduced in a degree corresponding to the volume sequestered by stasis in that dilated capillary stream bed.

Krogh showed that many agents cause capillary dilatation by direct effect. These include anoxia, heat, cold, light, a wide variety of chemicals, drugs, metabolites and substances of bacterial and animal origin. Most of these agents affect the permeability of the endothelium as well as the diameter of the capillaries and venules. This results in loss of tonus, dilatation and increased permeability to the plasma colloids. Capillaries so affected do not respond to the stimuli which cause normal capillaries to contract. These conclusions have been substantiated by other students of capillary physiology (Ebbecke; Lewis; Landis).

Lewis showed that any type of mild local injury to dermal cells produces a vascular reaction which he called the "triple response." This consists of: (1) local dilatation and engorgement of the capillaries and venules; (2) the formation of a circumscribed wheal consisting of

edema, which results from leakage of plasma through permeable endothelium; (3) a spreading flare due to reflex dilatation of the neighboring arterioles. He showed that cells respond to injuries of all types by liberating substances the effects of which on the minute vessels are like the effects of histamine. These H-substances produce dilatation and an increase in permeability of the capillaries in a zone adjacent to the injury. As set forth by Lewis the liberation of large amounts of H-substance, as following extensive wounds or burns, would affect the systemic capillaries in a similar way, resulting in dilatation, congestion, stasis and edema in visceral areas. He endorses the interpretation that substances so derived affect the systemic circulation. "These effects lead to an impounding of the blood in the capillary reservoir accompanied by a serious loss of blood fluids into the tissue spaces. Owing to this diversion of the blood the central vessels are depleted, a profound and lasting fall of blood pressure follows, leading to a condition of collapse."

A mild local burn results in liberation of H-substances locally and produces the "triple response." An extensive superficial burn causes liberation of H-substances in amounts sufficient to affect the minute vessels in systemic areas and is followed by the circulatory deficiencies characteristic of shock. Hemoconcentration develops, the blood volume decreases, and postmortem examinations of the viscera show evidences of vascular dilatation and permeability of the same type as occur about a small local burn.

Krogh (p. 355) regards it as "proved conclusively that traumatic shock is due to the action of toxic substances formed without the intervention of micro-organisms in the injured tissue and distributed throughout the body by the circulating blood itself. It is evident now that these substances belong to the class of H-substances." He believes that the collapse in the circulation following extensive superficial burns is due to the same mechanism (p. 356).

Ebbecke's studies on capillary reactions antedated those of Lewis, but the conclusions of these authors are essentially identical and mutually confirmatory. He showed that the local hyperemia resulting from any injury to tissues is independent of innervation and is produced by products released locally by cells that have been stimulated or injured. These products cause capillaries and venules to dilate and to become permeable to the plasma. This results in edema and in stasis of closely packed corpuscles in the minute vessels. Shock results whenever a similar condition is produced systemically. He drew a significant comparison between the capillary phenomena in wheals and in shock. If one understands the mechanism of traumatic wheals, histamine, peptone and anaphylactic wheals, one likewise understands shock produced by trauma or burns, histamine, peptone and anaphylaxis. They are expresssions of

identical capillary reactions, the one in local areas, the other in visceral or systemic areas. The difference between them is quantitative not qualitative.

It is recalled that the poisons of various marine animals will produce wheals if introduced into the skin. Richet showed that these produce death from failure of the circulation when given intravenously to dogs. Significantly he named these "congestins" because of the visceral appearances produced by their action. Landis' studies on capillary permeability indicate that any agent or condition injurious to capillary endothelium causes an increase in the permeability of the capillary walls. This makes it probable that many other agents than those mentioned may have similar effects.

EVIDENCE AGAINST TRAUMATIC TOXEMIA AS THE CAUSE

Beginning in 1927 Blalock and his collaborators published a series of twelve reports on experimental shock. The experiments were made on dogs and dealt with the production and treatment of hemorrhage and shock and with the associated physiologic disturbances. He found it impracticable to distinguish hemorrhage from shock, and the entire series of papers supported this thesis.

Shock was produced in each dog by trauma to the muscles of the thigh. The animal had been anesthetized by an intravenous injection of sodium phenobarbital (soluble phenobarbital U. S. P.)—0.3 Gm. per kilogram of body weight. Following death the posterior parts of the body were bisected with care, and the weights of the traumatized and untraumatized halves were compared. The difference in weight represented extravasated blood and serous fluid in and about the traumatized areas and averaged 3.66 per cent of the body weight. This was calculated as equivalent to an average of 35.4 per cent of the total blood volume. Control dogs died from failure of the circulation when corresponding amounts of blood had been withdrawn. It is noteworthy that several dogs died following much smaller hemorrhages and that in two instances death occurred with the dog under barbital alone, without trauma or loss of blood.

There was evidence of redistribution of fluids following trauma and following hemorrhage. The tissues about the injury had an increased content of water, and those elsewhere, particularly the muscles, had lost water.

The authors found no evidence of shock following the release of the obstructed circulation from traumatized areas They injected fluid from normal and from traumatized limbs into normal dogs. A prompt decline in the blood pressure, usually ending in death, was the result, as described in a previous section (Blalock, reference d, p. 603). Blalock considered these experiments unsatisfactory for reasons not clear to the

reader. The conclusion was drawn that shock can be explained satisfactorily as resulting from loss of blood and fluid at the site of the trauma. He saw no evidence indicating formation or absorption of substances from traumatized tissue which would affect the circulation systemically.

Similar studies were made on shock following burns and following manipulation of the abdominal viscera. The authors believed that the fluid lost in and about burned areas or from the membranes of the bowel following manipulation was sufficient to explain the failure of the circulation in these conditions without resort to the supposition that injurious substances were absorbed from the damaged tissues.

Phemister and his collaborators published several reports similar to those of Blalock and his co-workers. The weights of traumatized limbs were compared with those of untraumatized limbs. The difference in weight was regarded as indicating the amount of fluid lost from the blood. This difference ranged between 310 and 1,057 Gm. The average increase in thirty-eight such experiments was slightly more than the average volume of blood which, if withdrawn gradually, would cause death by hemorrhage in dogs of similar size. Hemoglobin estimations and erythrocyte counts regularly showed a marked decrease in the concentration of blood. In no instance did concentration of the blood occur.

Necropsy showed evidences of extensive hemorrhage. The heart and vessels contained little blood, and the organs and tissues were pale. There were extensive extravasations of blood, which infiltrated the septums and formed large collections in the torn and lacerated muscles. They concluded that the predominant factor in the failure of the circulation in these experiments was hemorrhage into the damaged tissues. They found no evidence indicating absorption of toxic substances from areas of injury.

In view of the losses of blood up to 1,057 Gm., the marked decrease in concentration of the blood, the anemic condition of the viscera and the massive hemorrhages found in the traumatized limbs, one must concur heartily in their conclusions. Any effects which might have resulted from absorption were so overshadowed by the effects of hemorrhage as to be unrecognizable.

Roome, Keith and Phemister reduced the blood pressure of dogs to "shock levels" by various methods and compared the effects of hemorrhage in each. Examinations of the blood showed hemoconcentration in shock produced by manipulation of the intestines, but following mechanical trauma to the muscles and following hemorrhage the blood was found diluted. This observation and the finding of very extensive hemorrhages in the traumatized limbs indicate beyond any doubt that hemorrhage was the major factor resulting from the trauma. Such

experiments provide no distinction between the effects of trauma and those of hemorrhage. They concluded that the effects of experimental trauma and of intestinal manipulation were due to local loss of fluid rather than to toxemia.

Freedlander and Lenhart performed experiments exactly similar to those of Blalock, Phemister and their associates. They endorsed the conclusion that shock following trauma can be explained as due to hemorrhage and local loss of fluid.

Harkins and Hudson produced shock by freezing one half of the dog's body with solidified carbon dioxide. This was followed by a marked fall in the blood pressure and by hemoconcentration. They found increased fluid in the frozen side, amounting to 2.55 per cent of the body weight, and believed that this amount of plasma if lost from the blood stream was sufficient to account for a large part of the shock in these animals.

Harkins made similar observations on shock produced in dogs by burning. Each dog, under barbital anesthesia, was encased in a plaster cast balanced on a tipping apparatus which recorded lateral shifts of weight kymographically. One side of the animal was then burned with a Bunsen flame applied through windows in the plaster cast. An immediate shift of weight to the burned side occurred. Hemoconcentration developed early, but the blood pressure did not decline until shortly before death. The body was then bisected longitudinally, and the weights of the two sides were compared. In six such experiments the burned side outweighed the other by from 1.5 to 3.1 per cent of the total body weight. Harkins concluded that the accumulation of fluid about the burned area was of importance and placed the reaction in the category of secondary shock. The inference that the failure of the circulation was due entirely to the local loss of fluid in the burned area is left to the reader.

Experiments of this type include a factor of error which escaped attention. As fluid escapes from the blood into the tissues of the affected side, fluid is simultaneously absorbed from the tissues of the normal side thereby decreasing its weight. Marked dehydration of uninjured tissues following injury accompanied by hemorrhage was noted by Blalock and his associates, by Parsons and Phemister, by Robinson and Parsons and by others. Suppose 100 Gm. of fluid were shifted by redistribution from one side to the other, the difference in weight of the two sides would then be 200 Gm., but the actual gain of the affected side would be only 100 Gm. The difference in weight in such experiments includes twice the volume of fluid shifted by absorption and redistribution. Such experiments provide no means for determining what amount of fluid is absorbed from the normal tissues by dehydration. The factor of error multiplied by 2 occurs in all such computations.

Roome and Wilson obtained fluid from traumatized muscle under great pressure in a hydraulic press. Sudden death followed intravenous injection of such fluid in each of six dogs. Muscle juice and fluid obtained in the same manner but freed from sediment and fat by centrifugation were injected into three heparinized dogs. This resulted in an increase in blood pressure in two and in a decrease of 36 mm. in one. When extract was injected into the same dog from which the muscle extract was derived, the blood pressure fell immediately 43 mm. In a second series, muscle juice combined with bloody fluid from the traumatized tissues was given to five dogs. A moderate increase of blood pressure resulted in each. The injection of bloody fluid alone caused a slight rise of blood pressure in four and no variation in blood pressure in two. The injection of muscle juice alone caused a fall in blood pressure in five dogs and no change in blood pressure in one. In each instance the variations in pressure were transient.

The amount of extract given to one dog was stated. No other data as to dosage accompany this report. It is known that small doses of some substances cause increases in blood pressure, while larger amounts cause the pressure to fall. It seems that data concerning dosage would be pertinent to such experiments. The bearing of the recorded results on the mechanism of shock is ambiguous. Their maximum apparent significance is that they confirm the previous observation (Vincent and Sheen; Bayliss; Carlson; Dale; Collip and others) that most tissues contain both pressor and depressor substances, one or the other of which may predominate, depending on the method of extraction.

Notwithstanding the limited number and varying results of these experiments, the authors interpreted them as invalidating the "toxic theory" of shock. More remarkably, this report and its conclusions were made the theme of an editorial in *The Journal of the American Medical Association* (see bibliography under "Traumatic Shock") renouncing other explanations and indicating "that the conditions of hemorrhage and shock are identical."

(To be concluded)

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Charles S. McCleskey, assistant professor of bacteriology at the State University of Iowa, has been appointed associate professor of bacteriology at the Louisiana State University.

Vincent J. Dardinski has been appointed full-time pathologist of Georgetown University Hospital, Washington, D. C.

The Trudeau Medal of the National Tuberculosis Association has been presented to Charles J. Hatfield, associate director of the Henry Phipps Institute of the University of Pennsylvania.

Russell L. Holman has been appointed assistant professor of pathology in the medical school of the University of North Carolina.

H. D. Bergey, formerly professor of bacteriology and hygiene in the University of Pennsylvania, died on September 5, aged 76 years.

In the school of medicine of the University of Oklahoma Francis C. Lawler has been appointed assistant professor of bacteriology and Onie O. Williams assistant professor of pathology.

Rufus Cole, director of the Hospital of the Rockefeller Institute for Medical Research since 1909, has retired on account of age. He will be succeeded as director by Thomas M. Rivers.

Alan R. Moritz, associate professor of pathology in Western Reserve University, has been appointed professor of legal medicine in Harvard University Medical School, succeeding George B. Magrath, who has retired.

Irvine H. Page, associate in the Hospital of the Rockefeller Institute for Medical Research, has assumed charge of the research department of the Indianapolis City Hospital.

John J. Andújár has been appointed assistant professor of pathology in the University of Arkansas.

George McLean Lawson, recently professor of public health and bacteriology in the University of Louisville, Ky., has been appointed professor of preventive medicine and bacteriology in the University of Virginia.

Thomas A. Gonzales, acting chief medical examiner of New York City since the death of Charles Norris in 1935, has been appointed permanently to the position by civil service examination.

Daniel Nicholson has been promoted to the professorship of pathology in the University of Manitoba, Winnipeg, in succession to William Boyd, now professor of pathology in the University of Toronto.

Clyde G. Culbertson has been placed in charge of the newly established division of laboratories of the medical center of Indiana University, Indianapolis. This division was created by the consolidation of the clinical laboratory of the medical center, the laboratories of the state board of health and a state medicolegal laboratory to be established.

Society News.—The American Society of Tropical Medicine will hold its thirty-third annual meeting at New Orleans from November 30 to December 3. The second Charles Franklin Craig Lecture on the subject "Tropical Medicine" will be delivered by George W. McCoy, medical director, United States Public Health Service, who will speak on "The History of Leprosy in the United States."

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

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Experimental Pathology and Pathologic Anatomy

Experimental Nephritis in Rats Induced by Injection of Antikidney Serum. J. E. Smadel, J. Exper. Med. **64**:921, 1936.

Nephritis can be induced in rats by injecting serum obtained from rabbits immunized with suspensions of perfused rat kidney. This antikidney serum contains a number of antibodies capable, on injection into rats, of inducing a severe anaphylactoid reaction with general vascular manifestations that involve the kidney as well as other organs. The serum also contains a nephrotoxic agent that affects the kidney primarily. The nephrotoxic effect is characterized clinically by severe persistent albuminuria with casts and, during the acute stage of the disease, transient anasarca, but no significant hematuria occurs. When a severe anaphylactoid reaction is imposed on the nephrotoxic injury, hematuria is an outstanding feature. Nephrotoxin is demonstrable in vivo and is not related quantitatively to the precipitins in the antikidney serum. It is most readily obtained by immunization with kidney suspensions but may occasionally appear after injections of other organ preparations; it does not result from immunization with erythrocytes or serum. Nephrotoxin is present in the globulin fraction of antikidney serum. The nephrotoxic agent of antikidney serum is easily removed by absorption with kidney cells or fat-free kidney tissue. Similar preparations of liver likewise remove it but less readily. Kidney, liver and brain lipids fail to affect it, and absorption with red blood cells or serum has no effect on it. Nephrotoxin appears to be an antibody that is relatively organ specific. It differs from the more common antibodies involved in reverse anaphylaxis in one respect at least. The animal rapidly becomes desensitized against the latter and fails to react, whereas desensitization to nephrotoxin is difficult to secure.

From Author's Summary.

Effect of Experimental Reduction of Kidney Substance on the Parathyroid Glands and Skeletal Tissue. A. M. Pappenheimer, J. Exper. Med. **64**:965, 1936.

Reduction of renal tissue in young rats regularly leads to marked increase in the volume of the parathyroid glands. If partially nephrectomized rats are maintained on a diet low in calcium, growth is stunted and skeletal lesions are produced which are of far greater severity than can be ascribed to the deficiency of calcium alone. The picture closely resembles that in renal rickets in children.

From Author's Summary.

Experimental Renal Hypertension. H. Goldblatt, J. Gross and R. F. Hanzal, J. Exper. Med. **65**:233, 1937.

In dogs, excision of the thoracic portion of the splanchnic nerves and the lower four dorsal sympathetic ganglions on each side does not prevent, cure or permanently lower to any degree experimental renal hypertension produced by renal ischemia.

From Authors' Summary.

EFFECT OF PREGNANCY AND OF FEMALE SEX HORMONES ON SYPHILIS IN EXPERIMENTAL ANIMALS. J. E. KEMP, J. Infect. Dis. **60**:32, 1937.

Although the number of animals involved in this experiment is small and the results are not absolutely convincing, it is nevertheless suggested by this and other experimental and clinical studies that while pregnancy appreciably inhibits syphilitic infection it is not the only factor responsible for the alteration in the course of this disease in the pregnant female. The experience of Frazier and his colleagues in treating experimental syphilis in rabbits with estrogenic substance prepared from the urine of pregnant women suggests that this hormone may be responsible for the modification of the infection in the pregnant female. Kemp's findings are not in agreement with theirs. Whether this is because the material they used contained a larger amount of estrogenic substance than he used, or because his material contained, in addition, an appreciable quantity of the anterior pituitary-like hormone or other substances, is not evident. It is evident, however, that further experimental and clinical studies are necessary before the relationship of sex and pregnancy to the course of syphilitic infection can be accurately determined.

FROM AUTHOR'S CONCLUSIONS.

THE BLOOD AND TISSUES IN NUTRITIONAL MUSCULAR DYSTROPHY. S. MORGULIS and H. C. SPENCER, J. Nutrition **12**:173, 1936.

Muscular dystrophy was produced in rabbits by feeding diet 13 of Goettsch and Pappenheimer. Analyses of blood obtained from the normal and the dystrophic rabbits during fasting showed no significant variation in sugar and lactic acid and in total and soluble phosphorus, but in the diseased animals there was a marked increase in the cholesterol and in the lipid phosphorus of the blood. The values returned to normal with the initiation of regeneration leading to recovery. The glycogen content of the skeletal muscles of the dystrophic rabbits was very greatly reduced, the reduction being proportional to the degeneration. The creatinine content of the muscle also decreased in proportion to the development of dystrophy, while the cholesterol content was greatly increased in all cases.

R. J. LEBOWICH.

EFFECTS OF ESTROGENIC SUBSTANCE ON THE PROSTATE AND UTERUS MASCULINUS IN VARIOUS SPECIES OF PRIMATE. S. ZUCKERMAN and A. S. PARKES, J. Anat. **70**:323, 1936.

Injections of estrogenic substance appeared to have no effect on the prostate of the marmoset and that of the capuchin. The most pronounced changes induced in the prostate in the common macaque, the green monkey and the mona monkey were stratification and cornification of the prostatic utricle and increased general fibromuscular growth. The true prostatic glands of the mona monkey undergo active and irregular epithelial proliferation. The distal part of the common ejaculatory duct of the green monkey shows epithelial proliferation. In the langur, there is disorganized glandular hyperplasia of the prostatic utricle in addition to changes in the prostate. The authors consider the clinical significance of the changes in the prostate of the langur in relation to the fact that epithelial metaplasia has not been observed in the true prostatic glands of monkeys during prolonged administration of estrogenic substance.

R. J. LEBOWICH.

METABOLISM OF JOINT TISSUES. E. G. L. BYWATERS, J. Path. & Bact. **44**:247, 1937.

The metabolism of synovial villi is similar to that of fibroblasts and osteoblasts, with relatively high glycolysis (compared with oxidative metabolism) and a respiratory quotient of from 0.71 to 0.72, while the metabolism per cell is of the same order as that of other adult tissues. Provided the synovial fluid is kept in equilibrium with the blood, the oxygen requirements of the villi can be met entirely from the joint fluid. From a small series of experiments it appears that the concentration of mucin in the synovial fluid varies directly with the glycolytic

activity of the fringes. The explanation may postulate secretory activity, but it seems possible that this association may be partly due also to an inflammatory increase of cells with an associated decrease in absorption of colloid (due to lymphatic obliteration). The concentration of mucin varies roughly with the viscosity. Articular cartilage shows active glycolysis, which is of the same order per cell as that of other adult tissues and is maintained, but at a lower rate, in the absence of dextrose. No certain difference between aerobic and anaerobic glycolysis is found either directly or with fluoride. The inhibition due to fluoride is less in the absence of dextrose. The oxygen uptake of cartilage is too small to be accurately assessed; it is immediately increased to a measurable size, probably about twenty times, on the addition of reduction-oxidation dyes. The presence of dehydrogenase and the absence of indophenol-oxidase are confirmed. The thickness of articular cartilage is estimated to be such that under normal conditions the deepest layers can be supplied entirely from the synovial fluid with those substances they have been shown to need. Under abnormal conditions, such as increased thickness of the cartilage or decreased permeability of the synovial membrane, this does not necessarily hold. A human enchondroma showed metabolism intermediate between that of cartilage and that of synovial membrane.

FROM AUTHOR'S SUMMARY.

ANASTOMOSIS OF THE PANCREATIC AND BILIARY DUCTS IN DOGS. J. BOTTIN, Arch. internat. de méd. expér. **11**:791, 1936.

An attempt was made to induce acute hemorrhagic pancreatitis in dogs by setting up the conditions postulated by Opie as pathogenic in human beings. The duct of Wirsung and the common bile duct were made to communicate, after which the common bile duct was obstructed distal to the point of communication. In no case was pancreatitis produced. A mixture of bile and pancreatic juice was found in both duct systems. The wall of the gallbladder commonly showed severe degenerative changes leading to biliary peritonitis with or without perforation of the bladder. No lesions certainly related to obstruction were encountered in the pancreas.

RALPH H. FULLER.

INFLUENCE OF ROENTGEN RAYS ON INFLAMMATION. W. G. GARSCHIN, M. M. BOLSCHAKOVA and V. V. OSSINSKAJA, Frankfurt. Ztschr. f. Path. **48**:131, 1935.

This article deals with the influence of roentgen rays on aseptic inflammation and the resulting morphologic changes. Special attention was directed to alterations of macrophages. The dosage employed was from 3 to 6 skin erythema doses. The aseptic inflammation was produced with silicon. The outstanding feature was the inhibitory effect on the growth and development of granulation tissue. The rays also affected the number of the cellular elements, reducing the number of macrophages and fibroblasts as well as the stroma of the granuloma. The irradiated granuloma showed a reduced number of giant cells, and those present were smaller and contained smaller nuclei. The collagenous fibers became markedly thickened. In some instances the inflammation disappeared, in other instances it was changed morphologically or so inhibited that foreign bodies became encapsulated. In chronic inflammation the radiation inhibited the development of the phagocytic elements. The dosage is an important factor in the production of the different changes. It was noted that 3 skin erythema doses administered at intervals of four days did not produce any noteworthy changes in the structure of the granuloma, while 3 such doses administered in half doses over the same time affected the granuloma markedly. This is probably due to summation of doses and to the lack of rest periods. Apparently no summation occurs when the doses are too small. Double doses were used over the same time and with the same intervals as previously mentioned, but no difference in the reaction was noted. Six doses produced only more marked fibrosis. No definite conclusions can be drawn as to the influence of fractional or full doses. However, the changes on the macrophages may aid in the evaluation of the phenomena produced by roentgen irradiation of inflamed tissues.

OTTO SAPHIR.

EXPERIMENTAL CAROTENOSIS AND THE YELLOW DISCOLORATION OF THE SKULL IN DIABETES. M. HARY, Frankfurt. Ztschr. f. Path. **48**:283, 1935.

To disprove the hypothesis that the yellow discoloration of the roof of the skull of the diabetic person is due to the presence of carotene and to substantiate the contention that it is caused by accumulations of fat, Hary fed yellow carrots to white rats for from twelve to fifteen weeks. Chloroform extracts of organs and bones of these animals were subjected to the sensitive fluorescence tests of Hoppe-Seyler and others and were found to have more or less yellow fluorescence, whereas the roofs of the skulls of diabetic persons had dark blue fluorescence, only the marrow cavities showing weakly yellow fluorescence. By this method Hary demonstrated the absence of carotene but obtained positive results in tests for fat in the skulls of diabetic persons. On the other hand, she could prove that hypervitaminosis A causes a disturbance in the fat threshold, producing accumulation of carotene with complete absence of fat in the skulls of experimental animals.

OTTO SAPHIR.

PRECOCIOUS SENESCENCE: A SEQUEL TO A CYST OF THE PINEAL BODY. G. KUP, Frankfurt. Ztschr. f. Path. **48**:318, 1935.

The pineal bodies of thirty-two patients with disturbances of internal secretory function were examined. In nine of these, alterations were noted, either the formation of cysts or parenchymatous hyperplasia. One case, reported in detail, concerned a man aged 25 who died of peritonitis following a stab wound. He had aged rapidly since the age of 18 and at the time of death appeared to be 40 years old. Replacing the pineal body was a cyst the size of a hazelnut. The testes were enlarged, and there was extensive atherosclerosis of the aorta and of peripheral vessels. Histologically, complete disappearance of the pineal body was noted, the cyst being lined by glia cells. The author believes that the pineal body of the adult plays an important rôle in the prevention of senescence and that hyperpinealism (glandular hypertrophy) causes obesity.

OTTO SAPHIR.

CHANGES IN THE TRANSPLANTED ECTOPIC FETUS. A. PONSOLD, Virchows Arch. f. path. Anat. **297**:425, 1936.

In ectopic pregnancy the fetus that escapes into the peritoneal cavity may undergo mummification, skeletonization or calcification (lithopedion). No satisfactory explanation of these varying end-results has been offered. To approach a solution of this problem experiments were made in which one fetus of a guinea-pig was transplanted into the peritoneal cavity of the mother animal from whose uterus it had been removed (autotransplantation). In each instance another fetus was removed and preserved for comparison later with the retained fetus; the undisturbed intra-uterine fetuses developed to full term and were delivered normally. In some experiments the free fetus alone was transplanted; in others, the intact membranes with their contained fetus. The transplanted fetuses varied in length from 3 to 10 cm., and in weight from 2 to 40 Gm. The periods of retention of the transplanted fetuses varied from 3 weeks to 1½ years. When the free fetus was transplanted, the omentum quickly became adherent to it, and the fetus was surrounded by a vascularized connective tissue membrane. The soft parts of the fetus were absorbed, leaving only the skeleton (skeletonization). When the intact sac with its contained fetus was transplanted, no adhesions formed between the sac and the surrounding tissues. The amniotic liquid was slowly absorbed; the fetus did not become macerated, but only partly dehydrated. The dehydration the author considers an early stage of mummification. Complete mummification and calcification did not occur, these processes evidently requiring a longer time than the maximal duration of the experiments.

O. T. SCHULTZ.

WAXY DEGENERATION OF MUSCLE IN CALVES DUE TO AVITAMINOSIS C. A. HJÄRRE and K. LILLEENGEN, Virchows Arch. f. path. Anat. **297**:565, 1936.

This work, from Stockholm, states that waxy degeneration of the skeletal musculature and of the myocardium is frequently encountered in calves in Germany, France, Switzerland and the Scandinavian countries. It occurs most often in the late winter or early spring and in years of poor harvest, when the food supply of livestock is qualitatively and quantitatively inadequate. Previously proposed explanations of the origin of the condition appeared unsatisfactory to the authors. Because of the peculiar pale appearance of the flesh, the condition is known as "white flesh" or "chicken flesh" or "fish flesh." The essential change in the muscles is identical with the waxy degeneration described by Zenker. Fat droplets appear in the degenerated muscle, and the latter becomes atrophic and may contain deposits of granular calcium. In the authors' own investigations of the disease, their observation of scorbutic changes in the teeth and bones led them to suspect that the condition might be due to a deficiency of vitamin C. Very young calves maintained on a diet low in vitamin C or on a diet low in both B and C revealed changes in the muscles, teeth and bones identical with those of the spontaneous disease. Lack of vitamin B seemed to have no part in the process, and the authors agree that beef animals are not susceptible to a deficiency of this vitamin. They conclude that "idiopathic" waxy degeneration of muscle in calves is due to a deficiency of vitamin C resulting from a low content of this vitamin in cow's milk at certain seasons and under adverse food conditions. Contrary to the generally accepted statement in the literature that beef animals are protected against a deficiency of vitamin C, they believe that up to 2 months of age calves are highly susceptible to a lack of this vitamin. They suggest that Zenker's waxy degeneration of muscle in man may be due to a similar cause.

O. T. SCHULTZ.

EXAMPLES OF DIFFERENT TYPES OF HERMAPHRODITISM. R. WEYENETH, Virchows Arch. f. path. Anat. **297**:594, 1936.

Klebs' older classification of hermaphroditism into true and false is no longer tenable. Weyeneth uses the more modern classification of Kolisko, which is as follows:

1. Hermaphroditismus externus.
2. Hermaphroditismus internus: (*a*) tubularis and (*b*) glandularis.
3. Hermaphroditismus externus et internus: (*a*) tubularis and (*b*) glandularis.

In some instances the true sex cannot be determined; for these the term "sexus anceps" is used. Weyeneth describes in detail seven examples of hermaphroditism and discusses the interpretation and bearing of his findings in detail. His material includes the following: hermaphroditismus masculinus externus, one case; hermaphroditismus femininus externus, one case; hermaphroditismus internus glandularis, three cases. In this type there is an ovotestis. The associated sexual characters may vary from male to female, depending on the predominance of testicular or ovarian tissue in the bisexual gonad. In one of the cases of this group an ovarian dysgerminoma had been previously removed. There was one case of hermaphroditismus internus tubularis in which there was a tubular adenoma of an abdominal testis. The final case was one in which the sex could not be determined grossly. It was not however an example of sexus anceps or even of hermaphroditism but of multiple malformations in a fetus with normal ovaries as determined by microscopic examination. The author confirms the finding of Mosckowicz that in feminine hermaphroditism the prostate lies cephalad and ventral to the vagina, and that in masculine hermaphroditism the prostate is penetrated by the vagina and the greater portion of the gland lies caudad to the vagina.

O. T. SCHULTZ.

EFFECT OF CEVITAMIC ACID ON FIBRIL FORMATION IN TISSUE CULTURE. A. VON JENEY and E. TÖRÖ, Virchows Arch. f. path. Anat. **298**:87, 1936.

The observation that cutaneous wounds of animals with experimental scurvy show little tendency to heal even after from seventeen to twenty-three days led to investigation of the effect of cevitamic acid on fibril formation in tissue cultures of fibroblasts. The vitamin added to the embryo extract was present in graded dilutions of from 0.000625 to 0.18 per cent. Fibril formation was most marked when the cevitamic acid content was 0.168 per cent. The authors conclude that the vitamin is necessary for the formation of a fibril-forming ground substance.

O. T. SCHULTZ.

BLOOD PRESSURE-RAISING SUBSTANCE IN CARCINOID. F. FEYRTER and K. UNNA, Virchows Arch. f. path. Anat. **298**:187, 1936.

The positive chromaffin and argentaffin reactions of carcinoids have led to the assumption that these tumors and the Schmidt cells from which the carcinoids are presumably derived are similar to the adrenal medulla. Proof of the assumption requires the isolation of epinephrine from carcinoids. Of extracts prepared from two appendical carcinoids, one had the characteristic blood pressure-raising action of epinephrine. The authors claim they have demonstrated the presence of epinephrine or, speaking more cautiously, of an epinephrine-like substance in carcinoids.

O. T. SCHULTZ.

Pathologic Anatomy

CYTO-ARCHITECTONIC ALTERATIONS OF THE BRAIN IN FATAL INJURY TO THE HEAD. C. W. RAND and C. B. COURVILLE, Arch. Neurol. & Psychiat. **36**:1277, 1936.

Architectural changes in the brain and cerebellum following injuries were studied by Rand and Courville in 229 cases of contusion and in 39 additional cases in which contusion was associated with laceration of the brain. In 18 other cases the injuries were represented by what the authors choose to call focal necrosis—acellular areas with or without hemorrhages—the condition being probably caused by "asphyxia" or "some unknown factors." Contusion of the brain, they think, is usually caused by contrecoup and may occur as a wedge-shaped superficial or diffuse lesion, depending on the location and intensity of the trauma. Whatever the type, cerebral contusion produces hemorrhages in both the cortex and the subcortex. In the areas immediately affected by the hemorrhages, the ganglion cells are destroyed; the adjoining area exhibits delayed disintegration and is followed by a zone of "reversible reaction," in which the ganglion cells, though injured, are capable of regaining their normal function. In "superficial" contusions, which occur mainly in the opercular region and the lower portion of the parietal cortex, there are streaked hemorrhages, with disintegration of superficial layers of the cortex. In the "diffuse" cortical contusion, which usually involves the temporal and occipital lobes, numerous focal hemorrhages are present in the cortex and subcortex and are associated with "disruption" and "disintegration" of the cortex, with apparent complete loss of ganglion cells. These, however, can be demonstrated with metallic staining. In the cerebellum, changes caused by injuries are of the same character as in the brain, the superficial folia being mainly involved.

G. B. HASSIN.

HISTOLOGIC STUDY OF MECKEL'S DIVERTICULUM. H. H. CURD, Arch. Surg. **32**:506, 1936.

Histologic study of 19 new cases of Meckel's diverticulum revealed a lining similar to that of the small intestine or a mucosa identical with that found in the stomach. Review of the literature shows 103 reported cases of Meckel's diverticulum with gastric mucosa. Gastric mucosa produces pepsin and hydrochloric

acid in umbilical polyps and fistula, as well as in Meckel's diverticulum. The acid gastric juice secreted undiluted into the intestinal canal may explain the occasional association of ulcers. The incidence of gastric mucosa in these diverticula was about 13 per cent. There have been 10 reports of the presence of duodenal glands and 35 of pancreatic tissue in Meckel's diverticulum. Every type of anomaly resulting from failure of all or part of the vitelline duct to become obliterated has been described. Failure of atrophy of the distal part of the duct results in an umbilical fistula or polyp. Failure of the whole duct to retrogress results in a patent umbilical fistula, and sometimes Meckel's diverticulum atrophies incompletely, a thin fibrous band resulting at the distal end. Gastric mucosa has been reported in other parts of the digestive system, as in the gallbladder, pancreas and various portions of the intestine. Inflammation, ulceration and cystic degeneration of parts of the intestinal tract occasionally have caused metaplasia of the epithelium, resulting in formation of gastric glands. The hypothesis suggested to explain the anomalous location of gastric tissue is that the primitive endoderm possesses the pluripotentiality of developing into several types of epithelium or glands of the intestinal tract and is locally stimulated to develop into a tissue anomalous for a particular region by trauma, infection or physiological-chemical change.

FREDERICK STENN.

PECULIAR GRANULES IN THE CELLS OF THE LIVER AND ADRENAL IN INFECTIONS. F. L. SANTEE, Bull. Johns Hopkins Hosp. **59**:427, 1936.

The purpose of this paper is to call attention to a neglected pathologic alteration in the cells of the liver and adrenal and to point out the relation of this change to infection. Patients who have died with severe infections frequently show at autopsy conspicuous oval or elongated granules in the cytoplasm of the cells of the liver and adrenal cortex. These granules are well stained with hematoxylin and with pyronine. It must remain for future work to determine their precise nature.

FROM AUTHOR'S SUMMARY.

BLOOD CYSTS IN HUMAN CARDIAC VALVES. D. R. DOW and W. F. HARPER, J. Anat. **71**:117, 1936.

Blood cysts were found in the valves of five of seven hearts of infants (up to 6 months of age). The specimens showed small dark red nodules projecting on the atrial aspects of the cusps, a short distance from their edges. They varied from 0.2 to 1 mm. in diameter and presented no external opening. One of the specimens contained microscopic cysts. The cysts consisted of monolocular spaces filled with erythrocytes and a few polymorphonuclear leukocytes and were lined with a single layer of endothelial cells. The tricuspid valves were most frequently affected. No pathologic effects were noted. Their origin is attributed to blood in the cavity of the heart being pressed into the cusps from the ventricular aspect.

FREDERICK STENN.

THE LARGE EXUDATE CELLS AND THE "EPITHELIUM" OF THE ALVEOLI OF THE LUNG. W. FIRLE, Frankfurt. Ztschr. f. Path. **48**:1, 1935.

Firle confirms the views of Maximow and concludes that the alveolar phagocytes are of histogenous origin (descendants of histiocytes) in noninflammatory processes and of a hematogenous origin (descendants of lymphocytes) in inflammatory processes. Since no epithelial or membrane-like alveolar lining was present, the belief is expressed that the alveolar lining consists of a fine network of blood capillaries supported by a framework of elastic fibers. The alveolar phagocytes are interpreted as specific elements and constitute a part of a defense reaction.

OTTO SAPHIR.

SYPHILITIC ENDOCARDITIS OF THE AORTIC VALVE. H. ŠIKL and K. RAŠKA, Frankfurt. Ztschr. f. Path. **48**:20, 1935.

Two cases are reported in which a relatively recent syphilitic inflammation with necrosis was found superimposed on old healed lesions of the aortic cusps. In both instances spirochetes were found in the aortic valve. In one case the old lesion was rheumatic, while in the other it represented bacterial endocarditis. Syphilitic aortitis was also present to a moderate degree in both cases. It is emphasized that the process interpreted as syphilitic was mainly localized on the free margin of the aortic cusps. The authors believe that the old bacterial and the rheumatic endocarditis had made the valves susceptible to the syphilitic involvement.

OTTO SAPHIR.

HEMORRHAGIC ESOPHAGITIS. K. NEUBÜRGER, Frankfurt. Ztschr. f. Path. **48**:105, 1935.

Neubürger reports seven cases in which at autopsy there was revealed a blackish brown discoloration of the superficial layers of the lower two thirds of the esophagus. The pigmented area was sharply demarcated from the cardia and extended by streaks toward the unpigmented area. There was no general intestinal melanosis present in any of these cases. There was likewise no history of poisoning with acids. In five cases multiple hemorrhagic erosions were present in the stomach, and in one case large and small prepyloric ulcers were seen. In another case the gastric mucosa was intact. In all instances generalized cardiovascular disturbances were present (in three cases cirrhosis of the liver; in one severe arteriosclerosis, and in the remainder damage of the heart muscle). Microscopic examination of the discolored area in the esophagus revealed destruction of the epithelial layer and replacement by clumps and masses of light brown to dark brown débris. Chemical analysis of these pigmented masses revealed hematin. Beneath this area was a layer of inflammatory cells. The muscularis mucosae was intact, but the submucosa presented fibrinous exudate. In one esophagus, the inflammation involved all layers, including the tunica adventitia. The epiglottis presented areas of necrosis, and the hypopharynx was infiltrated by polymorphonuclear leukocytes and lymphocytes. According to the author, this lesion had been present for about three days, since no signs of regeneration were noted. The changes are explained by the peptic action of the gastric juice (regurgitated by repeated attacks of vomiting), aided by the lowered resistance of the tissue. The acids of the gastric juice convert the extravasal and intravasal blood to hematin. Other factors in the pathogenesis may be general cardiovascular disturbances causing passive hyperemia. For the pathologic entity described, the author proposes the term "peptohemorrhagic esophagitis." Although this condition is of little clinical importance, it may be of medicolegal importance since it must be differentiated in some cases from acute poisoning with acids, which may give a similar picture. The author believes that in some cases gastric ulcers and erosions may be explained on the basis of the peptic action of the gastric juice in the presence of lowered resistance of tissue.

OTTO SAPHIR.

YELLOW DISCOLORATION OF THE SKULL IN DIABETES. K. THAISZ, Frankfurt. Ztschr. f. Path. **48**:418, 1935.

Thaisz was unable to demonstrate chemically or spectroscopically carotene in the skulls of diabetic patients but found large amounts of fat throughout the bones. Comparative tabulations of the amount of fat in the bones, the state of nutrition, the age of the patient and the symptoms of disease were made. It was not possible to correlate the state of nutrition with the fat content of bones, as greater accumulations of fat were observed in the bone cells of emaciated young persons than in those of the obese patients. A high fat content was consistently found in diabetes with acidosis, in marked anemia and in diseases causing disturbance of fat metabolism.

OTTO SAPHIR.

GUNSHOT WOUND IN THE HEART RESULTING IN SUBACUTE BACTERIAL ENDOCARDITIS. H. SIEGMUND, Frankfurt. Ztschr. f. Path. **48**:493, 1935.

A soldier who in 1917 suffered a wound in the chest from a hand-grenade splinter died in 1933 as a result of subacute bacterial endocarditis, which had been diagnosed six months before death. Autopsy revealed a scar in the left side of the chest, where the splinter had entered. It had passed through the anterior wall of the right ventricle and perforated the ventricular septum, the sinus of Valsalva of the right aortic cusp, the cusp itself and the aortic leaf of the mitral valve. Scars were present in all these regions. From the mitral valve, the splinter passed to the apex of the heart and became encapsulated in the region of the trabeculae carneae. On the aortic and mitral valves vegetations were present, superimposed on the scars. The author stresses the fact that those portions of the valvular endocardium which had not been in contact with the splinter were not involved by the endocarditic process. He also emphasizes the rarity of such a complication.

OTTO SAPHIR.

ENDOMETRIOSIS IN THE MUSCULATURE OF THE ARM. E. NAVRATIL and A. KRAMER, Klin. Wchnschr. **15**:1765, 1936.

An instance of endometriosis in the right Musculus extensor carpi radialis is described. The possible explanations for endometriosis at that point are discussed, especially the possibility of origin in loco from undifferentiated mesenchymal elements and of metastasis.

ORIGIN OF THE LEFT CORONARY FROM THE PULMONARY ARTERY. K. LINCK, Virchows Arch. f. path. Anat. **297**:113, 1936.

Anomalous origin of a coronary artery from the pulmonary artery may be the result of abnormality in the division of the bulbus arteriosus, in which case the coronary anomaly is usually associated with other maldevelopments. Or it may be the result of abnormality in the formation of the anlagen of the coronary arteries, in which event the coronary anomaly may be the only one noted. Origin of the right coronary from the pulmonary artery may be without effect if adequate anastomoses exist with the normal left coronary. Origin of the left coronary from the pulmonary artery may lead to serious disturbances in the nutrition of the myocardium because of the lower pressure in the artery, which supplies the greater part of the myocardium. In a girl aged 8 months, who died of bronchopneumonia, origin of the left coronary from the pulmonary artery was the only anomaly noted. It was associated with fibrosis of the myocardium of the left ventricle of a degree that one usually sees only in coronary arteriosclerosis of the adult. In an area the size of a mark-piece the wall of the left ventricle was completely fibrous as in a healed infarct; it was only 5 mm. thick, and in this region the wall of the ventricle had undergone aneurysmal dilatation.

O. T. SCHULTZ.

LEAD GANGRENE AND ENCEPHALOPATHY. E. RUTISHAUSER, Virchows Arch. f. path. Anat. **297**:119, 1936.

A man aged 68 years had had difficulty in walking and repeated attacks of intermittent claudication nine years prior to death. Gangrene of both lower extremities required four amputations in the course of the next three years. Histologic examination of the vessels of the amputated tissues revealed no arterial changes to account for the thrombosis, and embolism was excluded. At the time of the first admission mild diabetes was detected. Death occurred with symptoms of chronic nephritis and progressive paralysis. Necropsy revealed marked and extensive arteriosclerosis with calcification and ossification, malignant nephrosclerosis and a fragment of a lead projectile weighing 2.5 Gm. in the left occipital region of the brain. Later questioning brought to light a gunshot injury

some thirty-nine years prior to death. Quantitative chemical examination of the various organs, including the brain, showed the presence of large amounts of lead. Histologic examination of the brain yielded no evidence of syphilitic progressive paralysis but rather such changes as have been described in some cases of lead encephalopathy. Rutishauser's interesting interpretation is as follows: Chronic lead poisoning led to injury of the pancreas and mild diabetes. The gangrene of the lower extremities, without evidence at that time of arteriosclerosis, was the result of more manifest lead poisoning with vascular spasm. The onset of more active symptoms of lead poisoning after a latent period of thirty-nine years was due to mobilization of lead through the acidosis of the diabetic state. Continuing and progressive lead intoxication caused arteriosclerosis, nephritis and the degenerative cerebral changes of lead encephalopathy.

O. T. SCHLUTZ.

PERIARTERITIS NODOSA WITH MULTIPLE ANEURYSMS. K. W. WOLFF, Virchows Arch. f. path. Anat. **297**:177, 1936.

In a woman dead at the age of 33 years hypertension and abdominal tumor, probably paraganglioma, were diagnosed clinically. Necropsy revealed: multiple aneurysms of the abdominal aorta and of the renal, iliac, hypogastric, splenic and right subclavian arteries; marked periarteritic involvement of the kidneys with nephrosclerosis, and generalized secondary arteriosclerosis. On microscopic examination there were found in the various arteries of the body the lesions of periarteritis nodosa in all stages from the earliest to the latest. The fresher granulomatous lesions contained degeneration products morphologically similar to Russell's bodies. The formation of the aneurysms was the result of the necrotizing inflammation of the media that occurred in the periarteritis nodosa. Wolff thinks that the histology of his case offers support for Rössle's concept that periarteritis nodosa and thrombo-angiitis obliterans are allergic rheumatoid vascular diseases.

O. T. SCHULTZ.

RELATION OF THE BASOPHILIC CELLS OF THE NEUROHYPOPHYSIS TO HYPERTENSION AND ECLAMPSIA. K. SCRIBA, Virchows Arch. f. path. Anat. **297**:221, 1936.

A number of writers have ascribed the presence of basophilic cells in the neurohypophysis to invasion from the adenohypophysis and have seen in these misplaced cells the anatomic substrate for nonrenal hypertension and eclampsia. To the basophilic cells in the neurohypophysis is ascribed the property of producing antidiuretic and pressor hormones. Scriba examined serially the neurohypophyses of 346 subjects of both sexes and of all ages from the fetal period to beyond 80 years. The basophilic cells were found in fetuses and in children, as well as at all later ages, with no definite increase after the third decade. In general the cells were less numerous in women than in men. The 55 per cent of adults with numerous or fairly numerous basophilic cells were equally divided into those without and those with hypertension. The 45 per cent with few or no basophilic cells were again equally divided into those with and those without hypertension. Scriba therefore concludes that the basophilic cells of the neurohypophysis have no relation to hypertension and that no internal secretory activity can be ascribed to them. In children he observed groups of epithelial cells in the zone intermediate between the anterior and the posterior lobe of the hypophysis. These were derivatives of the posterior wall of Rathke's pouch. In 75 per cent of human beings such cells constitute a rudimentary organ analogous to the pars intermedia of the hypophysis of the lower animal; they have no endocrine function. It is these cells that become transformed into the basophilic cells of the neurohypophysis; the latter is not invaded by basophilic cells from the adenohypophysis.

O. T. SCHULTZ.

THE SUBENDOTHELIAL MESENCHYME OF THE ARTERIES IN TUBERCULOUS MENINGITIS. K. SPANG, Virchows Arch. f. path. Anat. **297**:264, 1936.

Vital storage experiments led Siegmund to conclude that the subendothelial tissue of the endocardium is part of the indifferent mesenchyme. To determine whether a similar tissue is present in peripheral arteries Spang studied the meningeal vessels in tuberculous meningitis. In vessels in which the adventitia is not yet involved by tuberculous necrosis there is present between the endothelium and the internal elastic lamina a cellular layer of variable thickness. The cells are histiocytes derived locally from the resting cells of the subendothelial indifferent mesenchyme.

O. T. SCHULTZ.

Pathologic Chemistry and Physics

THE CALCIUM CONTENT OF THE ARTERIOSCLEROTIC AORTA. S. R. HAYTHORN and F. A. TAYLOR, Am. J. Path. **12**:303, 1936.

From careful gross, microscopic and chemical examination of fifteen aortas which were selected according to the type of lesion present and compared, the following conclusions were reached: As the years advance, there is a consistent increase in the amount of calcium in the aorta which is independent of the type of lesion present. There is, in addition, an increase in the amount of calcium which depends on the amount of arteriosclerosis present and which varies with the kind and number of type lesions. The type lesions of arteriosclerosis may be classified separately for study, but each represents a step in a progressive disease and as such is rarely encountered singly in any given sclerotic aorta. The increase in calcium appears to depend on two processes: that associated with elastin in the media, and that depending on lipoidal degenerations in the contents of atheromatous cysts. The processes may operate singly, but as a rule they go on simultaneously. The highest calcium values were obtained for the calcium plates that appeared to be relatively stable end-products. Such plates were most commonly found in the abdominal segments.

FROM AUTHORS' SUMMARY.

MICROTECHNICAL DEMONSTRATION OF IRON. G. GÖMÖRI, Am. J. Path. **12**:655, 1936.

Hemosiderin does not contain ferrous compounds demonstrable with the direct Turnbull blue reaction. The alleged superiority of the Tirmann-Schmelzer modification of Turnbull's blue method is based partly on erroneous theoretical conceptions and partly on misinterpretation of artefacts. The best microtechnical reagent for iron is a mixture of equal parts of 20 per cent hydrochloric acid and 10 per cent potassium ferrocyanide solution. The time of exposure should be thirty minutes. Results of equal quality are produced by converting the ferrous sulfide obtained in Quincke's reaction into copper or lead sulfide. Both berlin blue and Turnbull's blue preparations can be made durable by diluting the Canada balsam to be used in mounting sections with old, oxidized oil of turpentine.

FROM AUTHOR'S SUMMARY.

DEPOSITION OF UREA IN THE BRAIN IN DIPHTHERIA. W. MÜLLER, Virchows Arch. f. path. Anat. **297**:141, 1936.

Examination of the brain in fifteen fatal cases of diphtheria by means of the xanthydrol reaction for urea revealed the presence of urea in such quantities as are usually seen only in extreme grades of uremia. There was not sufficient renal damage to account for so great a deposition of urea in the brain. Increased formation of urea resulting from the increased metabolism in diphtheria is held to be the main factor in the cerebral deposition of the substance.

O. T. SCHULTZ.

EFFECT OF HEAT INACTIVATION ON THE REACTIVITY OF SERUM. G. D'ALLESSANDRO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:154, 1936.

Unsaturated fats were extracted by ether at room temperature more readily and in larger amounts from fresh serum than from serum inactivated by heat. The total fat as determined by extraction with a mixture of hot ether and alcohol according to the method of Bloor was the same in fresh and in inactivated serum. Addition of small amounts of a phenol-alcohol mixture increased still further the yield of lipoids that could be extracted at low temperatures. Heating strengthens the lipoid-protein combination; phenol-alcohol loosens it. These observations are correlated with those of writers who found a noticeably greater readiness to give up fats in the tissues of rats with Jensen sarcoma.

I. DAVIDSOHN.

THE DEGREE OF SERUM INSTABILITY AND THE RESULTS OF LABILITY REACTIONS. F. HAHN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:252, 1936.

The lability reactions include the sedimentation of erythrocytes, the Takata-Ara reaction, the nonspecific complement fixation and flocculation with lecithin, cholesterol and extracts of lipoidal tissues, and a variety of other nonspecific reactions, all of which are based on a disturbance of the protein balance of the serum or plasma. They are all nonspecific, but some are quite frequent in certain diseases. According to Hahn, the different tests show the best reactions within a certain range of instability of the serum proteins. The nonspecific complement fixation with lecithin or cholesterol as antigen or rather as pseudo-antigen was particularly suited for mild degrees of serum imbalance. The Takata-Ara test and the erythrocytic sedimentation test were indicators of highest degrees of serum imbalance. The flocculation tests with cholesterol and lecithin as pseudoantigens had a much wider range of reactivity than the former. The reactions with all procedures differed in most cases in active and in inactivated serums.

I. DAVIDSOHN.

Microbiology and Parasitology

CHARACTERISTICS OF SMALL COLONY VARIANTS WITH SPECIAL REFERENCE TO SHIGELLA PARADYSENTERIAE SONNE. B. D. CHINN, J. Infect. Dis. **59**:137, 1936.

The G colony variant of Shigella paradysenteriae Sonne occurs infrequently and irregularly in aging agar slant cultures. Several strains were isolated by streaking nutrient agar plates from old cultures. The G colonies were generally very minute, and some reverted to the large colony form with difficulty. Some strains could not be brought back to the normal colony size. The cell morphology was irregular, and rods, cocci and filaments were present. The cell division of the variant differed somewhat from the normal, and reproduction was slow. The biochemical activity of the minute colony forms was greatly reduced. Sugars were fermented very slowly, and in some cases the ability to attack carbohydrates was lost. Methylthionine chloride (methylene blue) was reduced more slowly by the variant, and the rate varied with the size of the colony. Serologic tests showed some of the variants to be related to the normal. The G strains which reverted were agglutinated by normal antiserum to a high titer. By cataphoretic studies it was shown that the speed of migration of the variant was slower than the normal. Variants showing an intermediate colony size had velocities ranging between those of the normal and the variant. This indicated that the charge on the variant was lower. The variants, with one exception, were not filtrable through Berkefeld N and W candles or Seitz filters. One G colony form, which did not revert to the normal, passed through N filters about 50 per cent of the time.

FROM AUTHOR'S SUMMARY.

EPIZOOTIC STREPTOCOCCIC MYOCARDITIS IN GUINEA-PIGS. M. V. RAE, J. Infect. Dis. **59**:236, 1936.

The epizootic among guinea-pigs described manifested itself as an acute, rapidly fatal bubonic and septicemic condition affecting guinea-pigs of all ages and of both sexes. It was possible to reproduce in guinea-pigs and also in rabbits and mice, lesions identical with those of spontaneous origin. A fairly constant feature was acute nonsuppurative lymphadenitis without sinus formation. In the spontaneous disease the cervical glands were the site of predilection; in the artificially produced disease, the regional lymph nodes participated. Multiple necrotic foci in the myocardium formed a striking feature in the majority of cases, both spontaneous (80 per cent) and induced (94 per cent). The characteristic histologic picture in the myocardium embraced early edema and necrosis, the appearance of fibrin, bacterial invasion and a meager inflammatory cell response. Lymph nodes presented a granulomatous reaction with hyperplasia of the reticulo-endothelial cells. Necrosis of the liver was found in a few cases, but other organs were unaffected. The causative organism was an encapsulated hemolytic streptococcus, which was isolated from every case, usually in pure culture. Prolonged cultivation of the organism produced no change in its virulence, hemolytic properties, capsule formation or elective localizing propensity. FROM AUTHOR'S SUMMARY.

THE TRANSMISSION OF THE VIRUS OF INFLUENZA TO HEDGEHOGS. C. H. ST. HARRIS, Brit. J. Exper. Path. **17**:324, 1936.

It has been shown that the virus of influenza will produce a mild respiratory infection in the hedgehog. The condition is contagious and has been transmitted in series by direct inoculation through ten hedgehogs. Recovery from the infection is accompanied by development of antibodies in the blood, and the virus can no longer be recovered from the respiratory tract. There is no evidence that the hedgehog can have an inapparent infection or carry the virus for long periods. No opportunity has yet arisen to test the possibility of direct transmission of influenza from human beings to the hedgehog. Representatives of the primates, the carnivores and the rodents have been known for some time to be susceptible to the virus of influenza, and to these must now be added a representative of the insectivora—the hedgehog. It is of interest that influenza in the hedgehog resembles influenza in the ferret rather than that in the mouse.

FROM AUTHOR'S SUMMARY.

ANAEROBIC PNEUMOCOCCUS. F. SMITH, Brit. J. Exper. Path. **17**:329, 1936.

Two strains of anaerobic pneumococci are described. Serologically they are new varieties. One of the strains produces focal lesions in the kidneys of mice. The strains give rise to aerobic variants, and the variation is reversible.

FROM AUTHOR'S SUMMARY.

THE PERMEABILITY OF THE PLACENTA FOR VARIOUS MICRO-ORGANISMS AND BACTERIOPHAGES. T. NAKAGAWA, Zentralbl. f. Bakt. (Abt. 1) **136**:147, 1936.

Nakagawa injected various kinds of living bacteria, spirochetes, viruses and bacteriophages into the blood streams of pregnant rabbits and guinea-pigs and later examined the fetuses in order to see when and under what conditions injected materials appear in the fetus and amniotic fluid. He examined 700 pregnant animals and more than 2,000 fetuses. Pathogenic bacteria (streptococci, staphylococci, pneumococci and diphtheria bacilli) passed through the placenta within from two to nine hours; less pathogenic organisms, such as colon, typhoid and dysentery bacilli, went through scantily or not at all. Some bacteriophages passed through, and others did not. The placental permeability did not seem to depend on injuries to it or on pathologic changes but was influenced by such

factors as size of bacteria, motility and virulence. In fact, injury to the placenta seemed to delay the passage of bacteria from the maternal blood into the fetus. Nakagawa concludes that placental permeability is correlated with many complicated factors and that the process is not one of simple filtration but rather one of selective filtration.

PAUL R. CANNON.

THE VIRUS OF INGUINAL LYMPHOGRANULOMA. Y. MIYAGAWA and others, Jap. J. Exper. Med. **14**:197, 207 and 221, 1936.

The first article deals with the inoculation of the virus in small animals other than monkeys and mice. In the second article the results of the cultivation of the virus in tissue cultures are described. Such cultivation proved successful, and the virus thus maintained produced on intracerebral inoculation in mice typical symptoms and lesions. The cultures contained minute granules, both intracellular and extracellular and similar "granulocorpuscles" were found in the brain tissue of inoculated mice. The third article discusses the cultivation of the virus by the Tamura and Meyer-Enders methods.

TULARIMIA, A DISEASE OF LEMMINGS. E. SYLVEST, Ugesk. f. læger **98**:307, 1936.

For a long time it has been known in Norway that lemmings (Lemmus myodes) in their migrations may be attacked by disease and that simultaneously disease or diseases of infectious nature may occur in man. It seems not unlikely that the disease of lemmings and tularemia may be identical.

Immunology

MECHANISM OF IMMUNITY IN TYPHUS. M. R. CASTANEDA, J. Exper. Med. **64**:689 and 701, 1936.

The virus of Mexican typhus is capable of producing a local inflammatory reaction when injected intradermally into guinea-pigs. Rickettsias are easily found in the cutaneous lesion, particularly in the walls of capillaries, in places of little or no cellular reaction, in the early stages of the disease. The rickettsias are less frequently seen in places of increasing polymorphonuclear infiltration. In the mononuclear phagocytic nodules, characteristic of the lesion of typhus, rickettsias are rarely found. This may perhaps be due to an early destruction by polymorphonuclear phagocytes.

The intradermal inoculation of the virus into the immune guinea-pig produces a local reaction which is similar to that of the normal animal submitted to the same treatment. The reaction in the immune animal appears earlier and fades sooner than the lesion in the normal guinea-pig. It seems that the reaction observed in the immune guinea-pig submitted to a second inoculation of typhus virus belongs to the group of reactions presented by tuberculous animals (Koch's phenomenon) and to the accelerated takes shown by immune persons submitted to revaccination with the virus of vaccinia. A heat-labile substance has been demonstrated in the formaldehydized rickettsias, which produces a reaction in the skin of normal men and guinea-pigs.

FROM AUTHOR'S SUMMARIES.

SENSITIZATION OF ANIMALS WITH ARSPHENAMINE. K. LANDSTEINER and J. JACOBS, J. Exper. Med. **64**:717, 1936.

Experiments are described which show that with a given treatment guinea-pigs can be sensitized to arsphenamine so that a considerable percentage die in anaphylactic shock on intravenous administration of the substance.

FROM AUTHORS' SUMMARIES.

SOLUBLE PRECIPITABLE SUBSTANCES OF VACCINIA. J. CRAIGIE and F. O. WISHART, J. Exper. Med. **64**:803 and 819, 1936.

Thermolabile and thermostable soluble precipitable substances dissociate in vitro from the elementary bodies of vaccinia and occur in solution in suspensions of vaccine pulp. These substances participate in the agglutination of the elementary bodies by antivaccinial serum.

FROM AUTHORS' SUMMARIES.

IMMUNOGENETIC STUDIES OF SPECIES AND SPECIES HYBRIDS IN DOVES. M. R. IRWIN and L. J. COLE, J. Exper. Zoöl. **73**:85, 1936.

Rabbits were immunized with pooled bloods from representatives of the domesticated ring dove and with pooled bloods from representatives of an Asiatic species, commonly known as pearlneck. Each immune serum agglutinated the bloods of birds of both species in high dilutions. Whereas each serum could be completely exhausted by treatment with homologous blood, when it was absorbed by heterologous blood until it no longer reacted with such blood, it still agglutinated homologous blood in high dilutions. This indicated the presence in the bloods of these two species of doves of common species-specific properties and, in addition, factors peculiar to the separate species. Studies by absorption and agglutination were also made on the bloods of species hybrids (F_1) from male pearlnecks by ring dove females and on the bloods of quarter-pearlnecks obtained by back-crossing the hybrids with the domestic species. In this way the authors were able to demonstrate that the species-specific properties of the bloods of the two varieties of doves were determined by a large number of discrete agglutinable factors. The majority of these were evidently transmitted by a dominant heredity. However, the fact that anti-F_1 serum still reacted with the blood of all hybrids after they had been absorbed with both pearlneck and ring dove blood indicates the existence also of antigens with a recessive heredity. No attempt is made by the authors to estimate the actual number of agglutinable factors involved.

A. S. WIENER.

DETERMINATION OF BLOOD PROPERTIES M AND N. F. J. HOLZER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **26**:515, 1936.

Freshly ground liver, kidney and red blood cells of guinea-pigs, rabbits, cats, horses, pigs, monkeys, dogs and cows were added to equal volumes of solutions of agglutinins anti-M and anti-N and the mixtures allowed to stand over night. The following day the mixtures were centrifugated and the fluid separated. No significant change in the titers of the testing fluids occurred after this treatment. On the other hand, parallel absorption tests with homologous human blood of type M and type N showed a definite drop in titer even when quantities one fiftieth of the volumes of the agglutinin solutions were used. Hence the properties M and N are not demonstrable in the blood or organic tissues of animals.

A. S. WIENER.

IMMUNO-ISO-ANTIBODIES IN THE HOG. Z. SZIMANOWSKI and J. FRENDZEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:379, 1936.

A marked rise of iso-antibodies was observed in hogs that were given injections of the blood of other hogs. This took place when animals with normal anti-A agglutinins were given injections of blood A. In addition to a rise of the hog anti-A there was also a marked rise of the human anti-A and to a lesser extent of the human anti-B and anti-O agglutinins. The A factors of man and of hog have common features, as was shown by proper absorptions, but they are by no means identical. The O factors are species specific and not related; on the other hand, a relationship was noticeable between human B and hog O factors. A high titer of isolysins and of antisheep hemolysins was noted. The latter lysin fixed complement specifically with human A but not with hog A corpsucles. Such hog serums would lend themselves for the recognition of human A red corpuscles according to the method of Brahn and Schiff.

I. DAVIDSOHN.

THE RESISTANCE AGAINST TUBERCLE BACILLI AFTER IMMUNIZATION WITH DIFFERENT BACTERIA. S. NUKADA and C. RYU, Ztschr. f. Immunitätsforsch. u. exper. Therap. **88**:496, 1936.

The study was carried out on 1,333 male guinea-pigs and consisted in repeated inoculations of suspensions of killed bacteria and in some cases of horse serum or casein. Ten days after the last inoculation living and highly virulent tubercle bacilli were injected intravenously in a dose known to be fatal in twenty days. Vaccines of typhoid bacilli mixed with gonococci and of typhoid bacilli mixed with Bacillus abortus (Bang), vaccines of every one of the aforementioned species singly, and vaccines of Bacillus pestis, of Shiga dysentery bacilli, of paratyphoid A and B bacilli and of meningococci caused elevation of the resistance of the animals, evidenced by prolongation of their lives as compared with nonvaccinated controls. The degree of the raised resistance decreased in the order of the foregoing list of bacteria. On the other hand, inoculation with influenza bacilli streptococci, Bacillus pyocyaneus, staphylococci, Bacillus proteus, vibrios of cholera, Bacillus coli, Bacillus pertussis and pneumococci produced a drop in resistance. Horse serum and casein had no noticeable effect. Similar experiments on 203 rabbits showed similar results.

I. DAVIDSOHN.

INFLUENCE OF LECITHIN AND CHOLESTEROL ON THE HAPTENIC FUNCTION OF ALCOHOLIC EXTRACT. H. HAZATO, Ztschr. f. Immunitätsforsch. u. exper. Therap. **89**:1, 1936.

Cholesterol or lecithin added to heterogenetic haptens (alcoholic extract of the kidney of the horse) and to alcoholic extracts of beef heart, of beef brain and of human blood A influenced their ability to fix complement and cause flocculation with the corresponding antiserums. The influence was shown either in an increase or in an inhibition of these functions, depending on the rapidity with which the extracts were diluted with salt solution. Lecithin strengthened the antigenic properties of the fractional dilutions of the haptens, while in rapidly prepared dilutions its effect was inhibitory. On the other hand, the enhancing influence of cholesterol was strong only in the rapidly diluted haptens.

I. DAVIDSOHN.

AUTO-AGGLUTINATION. P. NEUDA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **89**:76, 1936.

Inactivation of an auto-agglutinating serum at 56 C. increases its auto-agglutinating property; incubation at 38 C. for twenty-four hours destroys this property and, in addition, makes manifest the blood group factors, which are not demonstrable in the inactivated serum. While this phenomenon is pronounced mostly in pathologic serums, it occurs in practically all serums and can be demonstrated by the finding of a marked rouleaux formation in the inactivated serum and by the absence of such a formation and an even distribution of the red cells in the incubated serum. Neuda refers to the studies of Kuerten (*Arch. f. d. ges. physiol.* **185**:248, 1920), who found that changes in the cholesterol-lecithin ratio of the blood serum affected the appearance of erythrocytes suspended in such a serum. According to Neuda, the conditions in a serum with an increase of cholesterol are comparable to those in the inactivated serum, and those in a serum with an increase of lecithin, to those in the incubated serum. Addition of inactivated serum inhibited the hemolysis by saponin and by lecithin, the agglutination of sheep erythrocytes by antisheep rabbit immune serums and of human A erythrocytes by anti-A rabbit serums. The cholesterol, a hydrophobic colloid, is kept in solution by lecithin, a hydrophilic colloid. Inactivation affects the colloidal relation of the two colloids and, according to Neuda, this change influences the serum-erythrocytes relation.

I. DAVIDSOHN.

ABSORPTION OF HEMOLYSINS AND AGGLUTININS. H. REPLOH and H. BOETTICHER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **89**:107, 1936.

The degrees of absorption of antisheep hemolysin and agglutinin from a rabbit immune serum with sheep erythrocytes were closely parallel, thus supporting the hypothesis of a unitarian nature of antibodies.

I. DAVIDSOHN.

THE PROBLEM OF THE HETEROSPECIFIC PREGNANCY. B. JONSSON, Acta path. et microbiol. Scandinav. **13**:424, 1936.

Qualitative estimations of the isolysins in 636 men of group O and in 594 women of the same group who had given birth to children a few months previously revealed a greater incidence of the lysin in the women; in addition, the women who had babies of group A showed a greater frequency of the anti-A isolysin, while women who had babies of group B showed a similar increase in the incidence of anti-B isolysin. Jonsson considers the antigenic action of the A and B isohemolysinogens in the babies responsible for the antibodies in the O mothers.

I. DAVIDSOHN.

Tumors

COMPARISON OF PROLAN BIO-ASSAYS IN TERATOMA AND OTHER CONDITIONS. S. E. OWEN and MAX CUTLER, Am. J. M. Sc. **192**:61, 1936.

Quantitative determinations of urinary prolan in 125 cases in which teratoma testis was suspected have been made. In general, patients with teratoma testis excrete more than 100 mouse units of prolan per liter of urine. Falsely negative or falsely positive results are obtained in less than 10 per cent of the cases; thus the test is as accurate as the usual laboratory test. A review of other conditions reported to be associated with excessive urinary output of prolan is presented. Original locally recurrent or metastatic teratoma is associated with increasing output of prolan, as determined by serial assays. Marked diminution in the output of prolan usually follows irradiation or surgical intervention.

FROM AUTHORS' SUMMARY.

EFFECT OF OIL OF WINTERGREEN ON SPONTANEOUS CARCINOMA IN MICE. L. C. STRONG, Am. J. M. Sc. **192**:546, 1936.

The daily administration of small amounts of the natural oil of wintergreen has had two effects on the incidence and severity of spontaneous carcinoma of the mammary gland. In the first place, its occurrence has been delayed to a later age, and, in the second place, the period of survival of the animal harboring it has been increased. This statement applies only to animals which were subjected to the treatment over a period of weeks or which were young mice at the start. The present study indicates that cancer of the mammary gland cannot be influenced by the daily administration of redistilled synthetic methyl salicylate through the diet when treatment is started after the onset of malignancy. The slowing up of the growth of the tumors in the treated animals is probably due to earlier mortality of mice in which the tumors were growing at a more rapid rate.

FROM AUTHOR'S SUMMARY.

A BIOLOGIC METHOD FOR STERILIZING CONTAMINATED TRANSPLANTABLE TUMORS. R. SCHREK, Am. J. Path. **12**:531, 1936.

The routine transplantation method used in this laboratory sufficed to sterilize a Flexner-Jobling tumor cell suspension purposely contaminated with Streptococcus viridans, and two contaminated Walker tumors. Sterile tumors were obtained by

transplanting once or twice minimal amounts (0.001 cc.) of the contaminated suspensions. The bacteria in the contaminated tumor cell suspensions caused a decrease in the percentage of takes of the tumor and prolonged the period of latency but had little if any effect on the growth rate of the resulting tumors.

FROM AUTHOR'S SUMMARY.

SEROLOGIC REACTIONS OBTAINED WITH A VIRUS CAUSING RABBIT PAPILLOMAS WHICH BECOME CANCEROUS. J. G. KIDD, J. W. BEARD and P. ROUS, J. Exper. Med. **64**:63 and 79, 1936.

A method has been devised for serologic tests with a virus producing rabbit papillomas that become carcinomatous. The discrete character of the growths when the virus is suitably diluted fits this virus notably for quantitative experimentation. The virus shows no tendency to lie latent in domestic rabbits, though it does so on occasion in cottontails, the natural hosts. Serums which partially neutralize it do not alter its character or attenuate it but merely cut down the number of its effective entities. The serum of normal domestic rabbits is ordinarily devoid of neutralizing influence on the virus, but that of animals carrying the papillomas usually exhibits neutralizing power soon after these appear. The rate at which this power increases depends in general on the amount of papillomatous tissue developing, but exceptions occur, the presence of fairly large growths being compatible with a lack of such powers in demonstrable amounts. Even when the antiviral power is great, it has no evident influence on the course of established papillomas, other factors determining whether these enlarge or retrogress. It acts to prevent successful reinoculation of the animal, however.

The serum of a rabbit with large cancers resulting from transplantation of a squamous cell carcinoma that had arisen from a virus-induced papilloma possessed the power to neutralize the virus, and so too in less degree did that of an animal of the same transplantation series in which a small nodule had developed. The serums of rabbits carrying tar papillomas or carcinomas of the Brown-Pearce type proved wholly devoid of effect on the virus. Implantation of Brown-Pearce tumor material mixed with virus did not lead to enduring establishment of the latter in the resulting growths or to any immediate changes in the morphologic character of these growths.

FROM AUTHORS' SUMMARIES.

THE RELATION OF THE VIRUS CAUSING RABBIT PAPILLOMAS TO THE CANCERS DERIVING THEREFROM. P. ROUS, J. W. BEARD and J. G. KIDD, J. Exper. Med. **64**:385 and 401, 1936.

The Influence of the Host Species and of the Pathogenic Activity and Concentration of the Virus.—All the strains of the Shope virus thus far tested, which give rise to vigorous, progressively enlarging papillomas in domestic rabbits, function as carcinogenic agents by way of these growths. The more pathogenic the virus, as evidenced by the brevity of the period of incubation and the vigor of the papillomas produced, the sooner and oftener does cancer occur. The number of virus entities contained in the inoculum notably influences the outcome, cancer appearing most frequently in those confluent papillomatous masses which have resulted from the greatest concentration of the virus material under test. The papillomas experimentally induced by the ordinary method of inoculation are essentially aggregates of proliferating cell families, each the outcome of some primary cell-virus association. Some of these associations are followed by cancer more frequently than others in the same animal. Cottontails, the natural hosts of the virus, are notably resistant to its sustained activity as compared with domestic rabbits. In most cases, the papillomas of cottontails, though often growing rapidly at first, soon became relatively inert, usually retrogress, and rarely undergo malignant change. In an instance reported here, both squamous cell carcinoma and metastasizing sarcoma appeared at the base of some papillomas produced by experi-

mental inoculation which had existed on the ears of a cottontail for nearly two years. The meaning of the phenomena is discussed.

The Evidence Provided by the Tumors: General Considerations.—The papillomas caused by the Shope virus sometimes spontaneously grow down into the subcutaneous tissue and extend along the lymphatics in the same way as does, in many cases, cancer of the human breast. They may, under such circumstances, invade the voluntary muscle, taking on an aspect suggestive of squamous cell carcinomatosis, but ultimately they differentiate in the way characteristic of the papilloma. Slight operative interferences with papillomas may be followed by development of secondary nodules in the lungs. These result from the lodgment of cell emboli, and the same local conditions determine their fate as are effective in the case of emboli composed of human cancer cells. The virus-induced papilloma is not only a neoplasm in its immediate aspect and habit but sometimes one that verges on malignancy. The tumors, including the cancers, which eventually derive from papillomas in favorable hosts, are representative of more than mere enhancement of growth. They develop within a relatively brief period but only after the papillomas have grown for a long while; and they are morphologically various whereas the parent tumors are remarkably constant in their form. Some of the new growths differ little from papillomas, however, even when possessed of the ability to metastasize and many continue to be influenced by the virus. The Shope virus is heavily conditioned in its carcinogenic activity, yet it is the nearest cause for cancer now known.

FROM AUTHORS' SUMMARIES.

TITRATION OF MOUSE TUMORS IN TRANSPLANTATION EXPERIMENTS. M. J. SHEAR, Pub. Health Rep. **51**:668, 1936.

Homogeneous tumor suspensions have been prepared by shaking minced tumor tissue in salt solutions containing gelatin. Mouse tumors have been titrated by injecting into pure strain mice equal volumes of tumor suspensions of varying dilution. With concentrated suspensions, "takes" were obtained in 100 per cent of the trials; with highly diluted ones, no tumors were obtained. Dilutions may be found with which tumors may be obtained in approximately half of the mice. When suspensions of tumors arising in mice of a pure strain were injected subcutaneously into mice of the same strain, sarcomas gave rise to tumors at high dilutions, whereas carcinomas gave rise to tumors only at comparatively low dilutions. Duplicate titrations gave results satisfactory as to reproducibility.

FROM AUTHOR'S SUMMARY.

REACTION OF BONE TO INVASION BY CARCINOMA. A. BRUNSCHWIG, Surg., Gynec. & Obst. **63**:273, 1936.

A study of metastatic carcinoma of human bone and of foci produced experimentally in the bones of rats showed that the new bone of intramedullary osteoblastic metastases is the result of two factors, namely, direct stimulation of the endosteal osteoblasts and infarction of the cortex and marrow, resulting in endosteal stimulation due to reduced circulation. Infarction may also cause aseptic necrosis of bone, and this is followed by creeping replacement of the dead bone by living tissue of the same kind. In any given case the two factors responsible for osteoblastic activity may vary in relative importance. Progressive elevation of the periosteum by accumulation of carcinoma cells beneath it acts as a stimulant to the periosteal cells and thus brings about new bone formation where osteoblastic metastases are supracortical in location. Nothing was found to support the hypothesis that new bone formation in either intramedullary or supracortical foci of osteoblastic metastasis is due to metaplasia of fibroblasts and other mesoblastic elements into osteoblasts, either within the marrow or in the soft parts in proximity to the cortex.

Osteolytic metastases appear to be due to the ability of some carcinoma cells to erode bone directly, stimulate widespread lacunar absorption and suppress the

normal osteoblastic activity of periosteum and "endosteum." At present there exists no explanation for the striking difference in reaction on bone by carcinoma cells as evidenced in osteolytic and osteoblastic metastases. The complexity of the question is made greater by the fact that both types may occur as metastases from the same primary growth or even occur side by side in the same bone.

WARREN C. HUNTER.

HYALINE CHANGE AND CALCIFICATION IN THE JENSEN RAT SARCOMA FROM THE INJECTION OF GELATIN, ACRIFLAVINE AND CALCIUM CHLORIDE. R. A. HUNTER, J. Path. & Bact. **43**: 35, 1936.

It has been shown that hyaline change and calcification can be regularly induced in the Jensen rat sarcoma by injections of gelatin, acriflavine and calcium chloride. These changes may occasionally occur in slow-growing or slowly regressing tumors. When induced in active, rapidly growing tumors, however, they tend to subvert growth. As there was no instance of complete retrogression when a gelatin and acriflavine medium alone was used, it appears that the combination of gelatin and calcium chloride is essential for the subversion of the neoplasm. By increasing the concentration of calcium chloride from 5 to 10 per cent, a more rapid destructive or inhibitory action on the tumor is obtained. Thrice-daily injections, with particular attention to the basal and marginal areas, would, it is believed, give even more satisfactory results.

FROM AUTHOR'S SUMMARY.

METASTATIC TUMOR OF THE BREAST. E. K. DAWSON, J. Path. & Bact. **43**:53, 1936.

Metastatic mammary tumors appear to be rare. Ten recorded cases are summarized, and a new one in which the primary growth was in the gastric mucosa is described. This tumor was of a mucus-containing, signet ring cell type; extensive carcinosis of lymph vessels was observed in the mammae, an ovary, the pancreas and lymph nodes, without the formation of obvious metastatic deposits in these organs.

FROM AUTHOR'S SUMMARY.

GENERALIZED LYMPHATIC CARCINOSIS OF THE LUNGS. T. T. WU, J. Path. & Bact. **43**:61, 1936.

Five cases of cancerous permeation of the pulmonary lymphatics is described, and the reports of forty-nine cases in the literature are reviewed. The stomach was the seat of the primary tumor in about three quarters of the cases. Less common sites were the bronchus, breast and prostate; rare sites, the uterus, sigmoid colon, gallbladder, ovary and tongue. The mechanism of lymphatic permeation and some possible etiologic factors are discussed. Obliterative changes of two types, thrombotic and endarteritic, were present in the pulmonary arteries in two of the author's cases. These he attributes to the effects of cancer cell emboli rather than to the mere presence of cancer cells in the perivascular lymphatics. Evidence in favor of this view is presented. The clinical manifestations of dyspnea and cyanosis, so frequent in these cases, may be due to various anatomic changes in the lungs resulting from the cancerous permeation of the pulmonary lymphatics. Some of the more extreme examples present the clinical features of Ayerza's syndrome.

FROM AUTHOR'S SUMMARY.

ANTAGONISM TO THE DEVELOPMENT OF MALIGNANT GROWTH IN TWO DIFFERENT ORGANS. W. CRAMER, J. Path. & Bact. **43**:77, 1936.

Experimental production of skin cancer by tarring in pure strains of mice having a high incidence of spontaneous mammary cancer yielded a percentage incidence of skin cancer not higher than that in mice with a low incidence of

spontaneous mammary cancer. This is in agreement with the results obtained by previous observers. On the contrary, there is evidence of definite antagonism for carcinogenesis in the two sites, skin and mamma. The skin of those individual animals in which mammary cancer most readily develops is definitely resistant to the carcinogenic action of tar. Conversely, individual mice in which the skin is most susceptible to the carcinogenic action of tar do not readily acquire mammary cancer. This constitutes further evidence that the carcinogenic response of the skin to a carcinogenic agent, although in itself a strictly localized process, is nevertheless conditioned by systemic factors lying outside the area subjected to the agent. As a result of this relationship, the simultaneous occurrence of skin cancer and mammary cancer is rare, and the incidence of skin cancer in tarred mice diminishes as the actual incidence of spontaneous mammary cancer increases. This may account for the statistical observation that different populations may show approximately equal total incidence of cancer in man with widely different incidence in organs.

FROM AUTHOR'S SUMMARY.

PRIMARY ENDOTHELIOMA OF THE PERICARDIUM. S. MCDONALD JR., J. Path. & Bact. **43**:137, 1936.

A primary malignant tumor of the pericardium is described, and reasons are given for regarding it as endothelioma. The literature of endothelioma of the pericardium is reviewed, and the histogenesis of this tumor is discussed.

FROM AUTHOR'S SUMMARY.

MALIGNANT HEMANGIO-ENDOTHELIOMA. R. F. OGILVIE and I. MACKENZIE, J. Path. & Bact. **43**:143, 1936.

Two cases of malignant hemangio-endothelioma are described, the first retroperitoneal in origin, the second originating in a cirrhotic liver. Tumors arising from vascular endothelium are considered as a whole, and it is shown that they can be classified in a series of increasing malignancy.

FROM AUTHOR'S SUMMARY.

TUMOR OF THE PITUITARY GLAND INDUCED WITH FOLLICULAR HORMONE. B. ZONDEK, Lancet **1**: 776, 1936.

Administration of follicular hormone to rats over a long period inhibits the anterior lobe of the pituitary gland so that the growth hormone and the gonadotropic hormone are not active. Dwarfed animals with hypoplastic genitals result. The dysfunctioning pituitary gland is enlarged, and the weight may amount to four times the normal. This increase occurs in male rats only. However, a very large tumor produced in the pituitary gland of a female rat was twenty times the normal size of the gland. This tumor produced signs of pressure on the brain and optic nerve. The pituitary glands of female rabbits do not enlarge after prolonged periods of treatment with folliculin. Whether or not this holds for male animals is now being studied.

FROM AUTHOR'S SUMMARY.

A STATISTICAL STUDY OF 2,179 TUMORS IN CHINESE. C. H. HU and K. Y. CH'IN, Chinese M. J., supp., 1936, p. 43.

Carcinoma and papilloma of the penis, carcinoma of the cervix uteri and carcinoma of the female breast are common, while carcinoma of the prostate, basal cell carcinoma of the skin and probably carcinoma of the stomach are either uncommon or relatively less frequent among Chinese than among Westerners. The probable causes of some of these differences are discussed.

FROM AUTHORS' SUMMARY.

ANTI-M AND ANTI-N SERUMS. T. KUSUMOTO, Nagasaki Igakkwai Zassi **14**: 133, 1936.

Rabbits were given injections of washed fresh human red blood cells of group O at four day intervals. O_M blood produced anti-M but no anti-N serum; O_N blood yielded anti-N but no anti-M serum; O_{MN} blood yielded one weak anti-M serum and several moderately strong anti-N serums. Seventeen rabbits were treated similarly with human serum; two weak anti-M and seven weak anti-N serums were obtained. Hence, human serum may occasionally contain the blood properties M and N.

A. S. WIENER.

Medicolegal Pathology

BLOOD GROUPING IN THE NEW YORK COURTS. A. S. WIENER, U. S. Law Rev. **70**:683, 1936.

Wiener presents his experiences with the application of blood grouping and M-N tests in cases of disputed parentage in the courts of New York since the passage of the law, in 1935, which gave the courts of that state authority to order blood grouping and receive the results in evidence. In a series of approximately 150 cases he was able to make a definite statement in 15 cases. In most of these cases proceedings had been instituted because a man accused of the paternity of an illegitimate child denied that he was the father. In one case the author was able to prove nonmaternity, thus revealing a fraud in which the woman had induced a certain man to marry her on the pretext that a child whom she had adopted was also their child. In another case four children of a married couple were found to be illegitimate. The author cites the passage of statutes in Wisconsin permitting courts to order blood grouping and predicts the ultimate widespread recognition and adoption of this test.

A. S. WIENER.

THE STABILITY OF CARBON MONOXIDE IN THE BLOOD. H. HÔDYÔ and S. WEHRLI, Deutsche Ztschr. f. d. ges. gerichtl. Med. **27**:111, 1936.

Experiments to determine the quantitative change in the carbon monoxide content of blood yielded the following results: The carbon monoxide content changes only slightly in years when the specimens are kept in sealed containers, even when marked decomposition is present. About half of the original carbon monoxide content was recovered from three specimens which were dry, and a sixth was found in a specimen kept in an open dish for thirty-three months. Generation of carbon monoxide did not occur in specimens of blood kept for as long as seventy-three days. Loss of carbon monoxide occurs when there is an exchange of this gas for atmospheric oxygen, and this may take place while a specimen is being transported for chemical analysis. Sedimentation may occur in blood containing the carbon monoxide-hemoglobin complex, and analyses of the serum may yield practically no carbon monoxide while the corpuscles are saturated. In order to avoid error in such instances, the whole blood should be saturated with carbon monoxide and then its original degree of unsaturation determined. Decomposition may bring about a marked increase in the carbon dioxide content—as much as 700 per cent.

GEORGE RUKSTINAT.

ACETIC ACID POISONING. W. NEUGEBAUER, Frankfurt. Ztschr. f. Path. **48**:222, 1935.

The toxic action of acetic acid is noted in seven cases which Neugebauer has observed and in cases collected from the literature. The acute changes produced by acetic acid consist in corrosion of the routes by which the poison enters the

body. Shock and immediate collapse following ingestion may lead to death as in mineral acid poisoning. Edema of the glottis and bronchopneumonia may also be the cause of death. Corrosion of the respiratory tract is due to the direct action of the corrosive poison. Absorption of acetic acid results in hemolysis, which characterizes the subacute type of poisoning. Nephrosis and hepatic damage may ensue. Such livers resemble those with so-called yellow and red atrophy. The centripetal distribution of the damage in the liver gives an appearance similar to that seen in association with hemolysis due to transfusion of incompatible blood. Therefore, the assumption seems justifiable that the damage in acetic acid poisoning is the result of hemoglobinemia. The author is of the opinion that acetic acid administered in the diet over a long period may become an etiologic factor in Laënnec's cirrhosis of the liver. Acetic acid damages organs through hemoglobinemia, and after absorption it may secondarily injure the parenchyma through hemolysis.

OTTO SAPHIR.

AUTHORIZED OFFICIAL NECROPSIES. R. RÖSSLE, Virchows Arch. f. path. Anat. **296**:535, 1936.

Rössle first discusses briefly the performance in Germany of what in this country would be termed "necropsies by permission." The German law neither requires nor prohibits necropsy of the body of a patient who has been under the care of a physician at home or in a hospital, provided there is no element of criminality in the patient's death. Usage has made postmortem examination in such cases customary in hospitals, and courts have held that because of such usage necropsy may be done without definite consent if the examination is not *prohibited* by the next of kin. Increasing use is being made of this right of prohibition by the laity, with the result that the number of necropsies in hospitals in Germany is decreasing. Rössle mentions some of the arguments against necropsy; they are much the same as those with which one has to contend in this country.

If there is any suspicion of a criminal element in the death, the matter must be investigated by the university medicolegal institute, which is a governmental agency. Rössle incidentally points out the relatively high cost of such investigations because of the wide variety of work the medicolegal institute must be equipped to do.

Rössle's main theme relates to the performance of necropsy in cases of nonviolent death in which it is found on examination that death was due to natural causes—such cases as in this country are reported to the coroner or medical examiner. They are chiefly cases of sudden death of those who have not been attended by a physician. In the more populous jurisdictions of this country such cases constitute approximately 50 per cent of the deaths referred to the coroner or medical examiner. In Germany, as in those parts of the United States where the coroner system prevails, cases of this type are reported to a nonmedical official, who may authorize a death certificate. Rössle deplores the vitiation of vital statistics that results from such a procedure and holds that the death rate from cancer in Germany for 1933—14.5 per ten thousand living—is from 20 to 30 per cent too high, whereas the number of deaths ascribed to alcoholism and syphilis is much too low.

In the category of sudden nonviolent deaths Rössle would place the death of any one who was attended by a physician so short a time that the latter could not reasonably be expected to make a correct diagnosis; the death of any one about whose condition prior to death the physician admits he is in doubt; the death of any one whose body is to be transported by rail or water—this as a public health measure; the death of any one whose body is to be disposed of by cremation (some of the states have such a law); any death due to an infectious disease or a disease suspected of being infectious, and any death involving insurance or compensation. He pleads that all such deaths should be referred to a medical official (in effect a medical examiner) who would be required to make a preliminary inspection. This official should be authorized to proceed to postmortem

examination if that is necessary to establish the cause of death. Rössle insists that this medical official should be an experienced pathologist. He believes that an official necropsy-service could be developed in Germany at relatively little cost by using as medical officials the pathologists of the university and hospital institutes of pathology. By automobile transportation a pathologist could cover an area with a radius of 60 kilometers. On a map of Germany Rössle has drawn circles with a radius of 60 kilometers about cities with university or hospital institutes of pathology. Practically the entire country falls within such areas except a part of east Prussia and a narrow strip along the Austrian border.

When one compares conditions in Germany as described by Rössle with those in this country, one comes to the conclusion that, with the exception of the work of the medicolegal institutes and a larger number of hospitals, the conditions there are just about as unsatisfactory as conditions under the coroner system are in the United States. Rössle speaks of the English coroner system as preferable to the system which prevails in Germany. The office of medical examiner as it is found in New York City, Boston and Newark approaches more nearly to what Rössle proposes for Germany, although the work in these cities falls short of the German medicolegal institutes because of inadequate financial support.

O. T. SCHULTZ.

Technical

SIMPLE SLIDE AND TUBE TESTS FOR INFECTIOUS MONONUCLEOSIS. R. STRAUS, Am. J. Clin. Path. **6**:546, 1936.

Straus offers two new modifications of the agglutination test for infectious mononucleosis. He uses a microscopic slide technic and a centrifuge method for the demonstration of heterophilic agglutinins for sheep erythrocytes. Titers from thirty-five controls, among them two from normal persons, are included. No controls are included for the centrifuge method. The advantage of the two modifications is the speed, as the time required after the dilutions have been prepared is only ten minutes. Three cases of active and three of borderline infectious mononucleosis are reported.

A MICROMETHOD FOR THE DETERMINATION OF TRYPTIC ACTIVITY. L. WEIL, Biochem. J. **30**:5, 1936.

Weil presents a method for the micro-estimation of trypsin in tissues by means of the apparatus of Linderstrøm-Lang and Holter. The formaldehyde titration method was used, with 4 per cent caseinogen as substrate at a p_H of 8.4 and with phenolphthalein as the indicator. The best condition for the reaction corresponded to the enzyme concentration which yielded a —COOH increase requiring < 7 cc. of five-hundredths-normal alkali. Tryptic activity was independent of the thickness of the section over the range of from 12.5 to 25 microns and of the time of extraction over from one to six hours. The error was found to be $\pm$ 0.1 cc. of five-hundredths-normal alkali.

R. J. LEBOWICH.

MICRODETERMINATION OF URIC ACID. N. L. EDSON and H. A. KREBS, Biochem. J. **30**:732, 1936.

Edson and Krebs describe a method especially suitable for work with tissue slices by which from 0.1 to 2 mg. of uric acid may be estimated with reasonable accuracy. The uric acid is converted first into allantoin, then into allantoic acid and finally into urea and glyoxylic acid. The determination is made manometrically with urease.

R. J. LEBOWICH.

RETICULOCYTES OF THE RABBIT. PIERRE NICOLLE, Arch. Inst. Pasteur de Tunis **25**:437, 1936.

A thorough study of normal rabbits in various stages of development indicated that supravital staining for the enumeration of reticulocytes might safely be replaced by hemolysis with dilute acetic acid and enumeration of the opaque elements. The method applies only to rabbits. It has many advantages, including rapidity of performance.

CRITICISM OF STERNAL PUNCTURE. K. HELPAP, Klin. Wchnschr. **16**:558, 1937.

Helpap's studies on the bone marrow of cadavers fail to substantiate the two factors on which the diagnostic use of sternal puncture is based: that the sternal marrow is homogeneous in its cellular structure and that a parallelism exists between the state of the sternal marrow and that of the marrow of other bones. He examined the sternal and the femoral marrow in thirty-two cadavers and found a homogeneous red marrow in only twenty-two. The cells obtained from each of three different places in the marrow of the sternum were different in eight cases. Examination of the removed sternum revealed many local irregularly distributed yellow areas in otherwise red bone marrow in six cases, and red irregularly distributed areas in otherwise yellow marrow in one case. In another case the sternum showed homogeneous red marrow in the manubrium, homogeneous gray marrow in the middle of the corpus, and red-yellow marrow in the lower third of the corpus. A lack of uniformity in the histologic structure of the marrow was also seen in both femurs. Thus, since the bone marrow is not a homogeneous organ, the diagnostic significance of the random sample of marrow is of doubtful value.

FREDERICK STENN.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, April 22, 1937

N. Chandler Foot, *President*

Milton Helpern, *Secretary*

Kaposi's Sarcoma: Two Solitary Nodules in Scalp and Forehead. D. S. D. Jessup.

A man of 65 years and of Italian birth applied for treatment at the tumor clinic. The family history was negative, as was the personal history, except that he had had an appendectomy twenty years previously.

Four months prior to consultation the patient noticed two small lumps on his scalp and forehead, close together. They were painless. The upper one had been operated on by his family physician, who had diagnosed a cyst. The operative wound failed to heal, and the lesions continued to grow. Examination showed on the scalp, over the forehead, just above the hairline, a pinkish red nodule, elevated, with a smooth, tense surface, measuring 2 cm. in diameter, and 2 cm. below it a similar slightly smaller nodule. Each nodule had a depressed center; both were indurated, but movable on the galea aponeurotica. A clinical diagnosis of basal cell carcinoma was made, and the two nodules were widely excised.

Pathologic examination showed two rounded growths similar in appearance and separated by 2 cm. of normal skin. There was slight ulceration of the upper one. On section the nodules had a depth of 7 mm., reaching down to the deep fascia and out laterally beneath normal skin, close to the line of excision at one point.

Sections showed a thin, flattened epidermis, and just beneath it and at the sides of the lesion there was a growth of vascular channels and sinuses, with proliferation of the endothelium and infiltration of the surrounding corium by large round and elongated cells, with areas of lymphoid cells. Farther toward the center of each nodule the proliferation of the blood vessels was accompanied by a growth of cells of fibroblastic type with some hemorrhage. There were also areas of large spindle-shaped and oval cells showing hyperchromatism, anaplasia and numerous mitoses. Scattered islands of new growth had spread into the surrounding derma, reaching the line of excision at one edge, and a large vein showed invasion by the growth, which nearly filled its lumen. The general picture indicated a malignant neoplasm, possibly metastatic, but physical examination, as well as blood and roentgen examinations, yielded negative results. After further study and consultation with Drs. F. C. Wood and James Ewing it was concluded that we were dealing with a sarcoma of the idiopathic hemorrhagic Kaposi type. The unusual location on the scalp without other manifestation of the disease seemed to be a feature of sufficient interest to warrant a report of the case.

Leiomyosarcoma of the Stomach Grossly Simulating Carcinoma. L. H. Meeker and (by invitation) M. H. Redish.

Sarcoma of the stomach is of sufficient infrequency to warrant presentation. It is in general seldom diagnosed correctly prior to operation.

A 52 year old white woman entered the New York Post-Graduate Medical School and Hospital for weakness and for pain in the back of four years' duration, which followed an attack of acute epigastric pain. Three months before admission

there was repetition of this abdominal distress. Roentgen examination revealed a large ulceration of the lesser curvature of the stomach. No anemia or essential gastro-intestinal symptoms were present. Physical examination showed nothing except marked pallor.

The clinical impression was that this was carcinoma of the stomach.

A Polya anastomosis and partial gastrectomy were made. A tumor, 10 by 8.5 by 7.5 cm., projecting from the lesser curvature and incorporated in the wall of the stomach, was removed. The removed tissues weighed 615 Gm. The patient died three days postoperatively in acute congestive heart failure.

The neoplasm was covered by serosa and was adherent to the under surface of the liver. Its bulk was excavated by a hemorrhagic, necrotic cavity from 15 to 40 mm. in diameter. The surrounding walls of firm gray tissue were marked by areas of cystic degeneration and hemorrhage. The central cavity communicated with the patent unobstructed gastric lumen through a 30 mm. ulceration of the lesser curvature. Elsewhere the gastric mucosa was intact.

Histologically the tumor cells were sharply defined from the overlying submucosa and mucosa. They reached out to, but did not invade, the serosa. The neoplastic cells were large, oval to round, with deeply staining vesicular nuclei. Mitoses were relatively uncommon. Areas of myxomatous degeneration and necrosis were present. The stroma was poorly cellular and highly vascular. Because of the highly anaplastic character of the tumor, together with its gross appearance, a tentative diagnosis of medullary carcinoma of the stomach was made.

At necropsy, several small gray metastases were found scattered in the liver. Microscopic study of these revealed the true nature of the malignant change. Here, the cells were arranged in nests and whorls and lay in a hyaline myxomatous stroma. The nuclei, generally oval to round, had gently rounded poles. A few of the cells assumed bizarre shapes or fused to form giant cells. Further study of the original tumor at this time showed the site of origin to be the internal layer of the muscular coat of the stomach.

This case emphasizes the almost universal failure of correct preoperative clinical diagnosis of sarcomas of the stomach, as well as the difficulties encountered in the anatomic recognition of these neoplasms.

DISCUSSION

PAUL KLEMPERER: In the differential diagnosis of sarcoma of the stomach one always has to consider neurogenic sarcoma, which occurs in the stomach quite frequently and shows a like peculiar cystic degeneration. I have seen two of tremendous size which were almost completely cystic. The differential diagnosis is sometimes very difficult.

WARD J. MACNEAL: I saw this patient at the operating table, and I think at that time there was no one who was willing to make a diagnosis of sarcoma. Most of us were of the opinion that we were dealing with adenocarcinoma of the stomach. It was adherent to the liver, and the question of operability was considered; the surgeon, after a great deal of discussion, undertook the removal, with the fatal result reported. I am especially interested in this presentation because Dr. Redish, Dr. Meeker and I now have a patient under observation who is still in the clinical stage, who has suffered from disease of the stomach for about a year and has been subjected to laparotomy by two eminent surgeons of this city with the diagnosis of inoperable carcinoma, but without biopsy, unfortunately. The patient remains at the end of a year well nourished, without any obstruction whatever, and eats well, with a very good appetite. The principal difficulty is persistent hemorrhage, with loss of blood in the stools and by vomiting. I believe Dr. Redish is inclined to think that this is another case in the same category.

The patient is now receiving very heavy roentgen therapy, the ultimate result of which may be considered to be doubtful.

TWO CASES OF GRANULOSA CELL TUMOR OF THE OVARY. L. H. MEEKER and (by invitation) S. A. LOCALIO.

A group of ovarian tumors capable of producing profound effects on the secondary sex characters of the hosts has been recently brought to our attention by Robert Meyer and Emil Novak. The most common and perhaps most important neoplasm of this group is the granulosa cell tumor. A resurvey of old material has resurrected many cases formerly classified as cases of sarcoma, carcinoma or endothelioma; at present there are about 200 properly designated instances reported in the literature. The incidence is from 10 to 14 per cent of primary malignant ovarian neoplasms.

According to Meyer's generally accepted theory, the origin of this tumor is related to persistent unused forerunners of the follicular epithelium. It is not surprising, therefore, that these tumors secrete estrogen and that the symptoms are secondary to an excess of this substance.

These tumors vary in diameter from a few millimeters to many centimeters. They may be smooth or lobulated, cystic or solid, and are often light yellow-brown. The extreme variability of the microscopic architecture has caused confusion. The tumors may assume folliculoid, cylindroid, mixed or sarcomatous patterns. The Call-Exner bodies are diagnostic. The degree of malignancy is in most instances low, and recurrences are exceptional. Novak prefers, therefore, to speak of them as granulosa cell adenomas; however, in from 5 to 10 per cent of the reported cases the growth is distinctly malignant and recurs.

CASE 1.—A white woman 22 years old was operated on in 1932 for intestinal obstruction. At operation the pelvis was entirely normal. The second admission was in August 1936, for menorrhagia and metrorrhagia of four months' duration. Examination under anesthesia revealed no pelvic changes. Curettage showed moderate endometrial hyperplasia and an excessive estrogenic reaction. Bleeding recurred three months later and continued for one month thereafter. Pelvic examination then revealed a firm rounded mass, thought to be a fibroid of the uterus. Histologically the endometrium was similar to that obtained at the previous curettage, and at laparotomy an encapsulated solid yellow-brown ovarian tumor, 8 by 7 by 5 cm., was found. Microscopically the tumor consisted of cylindromatous masses of small cells, varying in shape from round to polyhedral with large dark-staining to vesicular nuclei, suggesting follicular epithelium. Typical Call-Exner bodies were present. The patient, now in her fourth postoperative month, has remained symptomless.

CASE 2.—A 70 year old white woman noticed uterine bleeding fourteen years after menopause and six months prior to admission. This occurred each month, and the bleeding lasted from three to four days. The last episode persisted for eight days. Carcinoma of the uterus was suspected, but curettage revealed hyperplasia of the endometrium. The patient remained well for a short time; then bleeding was reestablished. An exploratory operation was performed, and a fibroid uterus and an ovarian cyst were removed. Microscopically the encapsulated, partially cystic yellow-brown ovarian tumor, 13 by 7.5 by 5 cm., presented a folliculoid pattern, with single circles of granulosa cells about small clear areas, which presumably contained estrogen. The patient, now in her fifth postoperative year, has remained symptomless.

Study of cases similar to the last one has shown that when a patient beyond the menopause exhibits menstrual or pseudomenstrual bleeding and curettage has ruled out carcinoma, granulosa cell tumor should be suspected. If the endometrium shows true hyperplasia and an ovarian tumor is palpable, the diagnosis is certain.

MALIGNANT MESENCHYMAL HEMENDOTHELIOMA. S. M. RABSON

This article will appear in full in a later issue of the ARCHIVES.

DISCUSSION

PAUL KLEMPERER: This is an interesting case, and I have had the privilege of seeing some of the slides. I have been particularly interested in the question of the multiplicity of the tumors. I think one can hardly doubt that some of the nodules must be metastatic. In this case it is easier to exclude multicentric origin than in other cases, because there were two enormous tumors, which one has to regard as the primary tumors. In other cases of a similar nature with widespread lesions it is more difficult.

My colleagues and I observed some months ago a case of Kaposi's sarcoma with most extensive involvement of the retroperitoneal tissues, the growth extending into the adrenals, into the spleen, around the pancreas and into the liver. The sarcomatous growths seemed to have originated in a lesion in the skin of the lower extremity. I say *seemed* because this was the first lesion appearing, but from the course of the disease it was evident that shortly after the appearance of these nodules there was also involvement of the inguinal nodes.

In regard to these mesenchymal tumors, a point of great interest is that one is hardly able to make a diagnosis of sarcoma, and, on the other hand, these tumors are definitely malignant. The first case seen by me was encountered in the Post-Graduate Medical School and Hospital, and Livingston and I published a report on it. The interesting thing was the histologic similarity of the first biopsy specimen to granulation tissue, which made me err in the diagnosis, because I received only a small particle, and this I diagnosed as granulation tissue. Only later sections made from more abundant material revealed that this was not granulation tissue in the sense of reparative mesenchymal tissue, but primarily tumor, and in these sections there were also areas of definitely malignant mesenchymal tissue.

A tumor I saw some years later, which belonged to the same form of multicentric mesenchymal tumors of a malignant character, having first the appearance of a benign lesion, was diagnosed as embryonal lipomatosis. The first tumor developed in the knee joint, which was diagnosed at that time by Dr. Theodore Mandelbaum as an ordinary lipoma, and reexamination of the slide showed that there was hardly any question about the simple lipomatous nature. This tumor recurred three years later, and again a diagnosis of lipoma was suggested, but on account of the recurrence of the tumor the diagnosis was changed to liposarcoma. Subsequently there developed on this site and in many other places multiple tumors of lipomatous character which, however, on histologic examination proved to be embryonal fat tissue, and while one could not make the diagnosis of liposarcoma, one had to make the diagnosis of embryonal lipomatosis, or, as I prefer, of a malignant mesenchymal tumor which appears under the various forms of the developmental potentialities of the mesenchyme—in this case, embryonal lipoma. In the case observed by Dr. Rabson the diagnosis of sarcoma is suggested, but, on the other hand, the presence of the variegated mesenchymal tissue suggests that one should not speak of sarcoma in the sense in which one commonly speaks of it, which means merely a tumor of very cellular character. I think tumors of the type described are not so extremely rare. Most of them are diagnosed as myxosarcoma, but their immature, undifferentiated mesenchymal nature and their multiplicity force one to separate them from the better known forms of sarcoma.

N. CHANDLER FOOT: It may possibly confuse the issue, but about two years ago Osrós, now in Budapest, published a paper in which he reviewed a number of tumors similar to these, in which there was a great deal of vessel sprout formation, under the name of gemmoma, or vessel bud tumor. The trouble with his classification was, I think, that it did not go quite far enough, because it would not cover such tumors as this one which Dr. Rabson has reported. There is no doubt that there is a good deal of difference between such tumors and the ordinary hemangiomas of the hamartoma type.

INTERRELATIONSHIPS OF THE OSTEOGENIC TUMORS. SHELDON A. JACOBSON.

In this schema, hereditary multiple exostosis is considered to be a dystrophy having its origin in metaplastic periosteal activity. (Nonhereditary multiple exostosis is a doubtful entity.) Nonhereditary multiple enchondromatosis is likewise considered to be a dystrophy, the result of failure of resorption of the provisional cartilage of the bones.

Osteochondroma occurring after the cessation of the usual period of growth should possibly be considered a true benign neoplasm, like osteoma (ivory exostosis) and enostosis.

The level of skeletal growth and of general metabolic reactivity drops from its high point at the epiphysial growth plates through the epiphyses proper and round bones of wrist and ankle to its minimum in the midshaft region. This is illustrated by the variation, in the direction and order named, of growth and reaction to such diseases as rickets, hunger osteoporosis and hyperparathyroidism. It is my belief that osteogenic sarcoma in the metaphysis, giant cell tumor in the epiphysis and osteoid-osteoma in the carpus or the tarsus represent similarly varying forms of the same neoplasm. They contain the same elements, differing only in malignancy.

Chondroma and chondrosarcoma may arise wherever cartilage exists—in perichondrium or the substance of costal cartilages, the symphysis pubis, etc., in periosteum, in the cartilaginous caps of exostoses, in preexisting enchondroma and in epiphysial cartilages. Whatever their site, the tumors are essentially the same, and any of them may demonstrate any grade of malignancy in architecture or in behavior.

Regular Monthly Meeting, May 27, 1937

N. CHANDLER FOOT, *President*

MILTON HELPERN, *Secretary*

ABSENCE OF THE SEPTUM PELLUCIDUM. VERA B. DOLGOPOL.

Absence of the septum pellucidum without other anomalies of the brain was observed in the brain of a woman of 61 who died of generalized arteriosclerosis.

A single ventricular cavity was present anteriorly; the fornix was not adherent to the corpus callosum. The large single anterior ventricle divided posteriorly into paired posterior and inferior horns. A moderate internal hydrocephalus was present, with no obstruction in the aqueduct or in the fourth ventricle, and was explainable on the basis of arteriosclerosis with atrophy of the white matter.

The case was that of secondary atrophy of a preformed septum pellucidum (Hochstetter) and not that of aplasia of this structure. The septum was fetal in its configuration at the time of absorption, as evidenced by the lack of adherence of fornix and corpus callosum. The time when the malformed septum pellucidum disappeared could not be established beyond the fact that the septum disappeared long before death. If any remnants of the septum were present at the time at which the senile hydrocephalus developed, the latter condition could contribute to their final resorption.

DISCUSSION

JAMES R. LISA: Recently my associates and I have examined a brain somewhat similar to this which just came to autopsy at the New York City Hospital. There was a single ventricle with complete absence of the septum. In this case it seems to be a congenital anomaly, for there is intercommunication between the vessels, which can be seen in the gross specimen. They run right across the midline, so apparently it is a congenital rather than an acquired anomaly. In this case there were no mental symptoms. It was the case of an adult dying of cardiac disease.

A Case of Bacteremia Due to Baccillus Welchii, with Spontaneous Extrusion of the Completely Infarcted Spleen Following Appendectomy with Recovery. S. H. Polayes and (by invitation) M. N. Foote.

An Italian boy 12 years of age was admitted to the Cumberland Hospital, Brooklyn, to the surgical service of Dr. M. N. Foote, with the complaints of pain in the lower part of the abdomen and vomiting. His past history was irrelevant. On physical examination a diagnosis of acute appendicitis was made, and an appendectomy through an incision in the right rectus muscle was carried out on the day of admission. At operation, in addition to acute, partly gangrenous appendicitis, there was also encountered a huge enlargement of the spleen, a small section of which was removed and showed necrosis of the splenic tissue (probably infarction) and clumps of bacilli, suggestive of a gas bacillus infection. Anaerobic blood and wound cultures were then made, and Welch's bacilli were recovered from each. Guinea-pig experiments, in which protective serums were used, confirmed the bacteriologic findings.

On the first suggestion of a possible Welch bacillus infection intensive antiserum therapy was instituted, and the patient made a remarkable recovery.

About six weeks after the appendectomy, following several days of fluctuation in temperature, a mass measuring 13 by 7 by 3 cm. was spontaneously extruded through the incision in the right rectus muscle, which had remained open since the appendectomy. Although the mass was extremely disintegrated, routine and special (Van Gieson, Weigert and Bielschowsky) stains of the tissue revealed its structure to be consistent with that of spleen.

The patient made an uneventful and speedy recovery after the sequestration of the mass and has enjoyed good health to date. No record of a similar case could be found in the literature.

DISCUSSION

Milton Helpern: Where in the abdomen was the spleen seen by the surgeon at the time of the biopsy?

S. H. Polayes: At the time of the operation the spleen was described as being so huge that it occupied the major portion of the left upper and a portion of the left lower quadrant. The biopsy was done on the right side of the midline.

Endocardial, Arterial and Other Mesenchymal Alterations in Man Associated with Serum Disease. Eugene Clark and (by invitation) Bernard Kaplan.

This article was published in full in the October issue of the Archives of Pathology, page 458.

DISCUSSION

Alfred Plaut: I should like to ask whether the lesions in the small arteries were diffuse or circumscribed, because focal lesions somewhat similar to those described by Drs. Clark and Kaplan have been found in a large number of vermiform appendixes, inflamed and noninflamed. My associates and I showed these five years ago at the meeting of the American Association of Pathologists in Philadelphia. Focal arteriolitis is found without relation to any disease, and my idea is that it might be a symptom of minor immunologic disturbances in the body, which in the major form were just demonstrated by Dr. Clark. I also wonder whether the changes demonstrated in the endocardium might occur more frequently in a much lesser degree. Occasionally I have seen in the endocardium accumulations of cells, and I discarded the observation because I had nothing to support a definite diagnosis. It is possible that minor immunologic disturbances might lead, perhaps not very infrequently, to minor changes in the histiocytes of the endocardium or other parts of the body.

It was interesting to me to see that the perirenal tissue and the testicle seemed to be preferred sites for the arteriolitic lesions. I have seen patients with infectious diseases who had necrotizing arteriolitis especially in these two locations.

SYLVAN E. MOOLTEN: It may be of interest to mention a case of meningococcic meningitis associated with periarteritis nodosa which I observed in 1935. The patient, a girl of 8, had been drowsy for three days, and then had convulsions, headache and abdominal pain with loose, blood-streaked stools for four days. The spinal fluid had a high cell count and showed many meningococci. The blood culture was negative. After 210 cc. of antimeningococcus serum had been given, all during ten days, her neurologic signs abated except for strabismus of one eye. During a protracted convalescence she still complained of abdominal pain, often severe, and the stool was blood stained. Three weeks after admission she began to show signs of diffuse peritonitis and passed blood clots. The association of antecedent acute infection and symptoms of intestinal infarction led me to suggest the diagnosis of periarteritis nodosa. This was confirmed at operation, in which over a meter of gangrenous ileum was removed. Throughout its length was found extensive acute and subacute necrotizing arteriolitis. The patient died very soon afterward. The vascular lesion was found to be more or less generalized at autopsy, the most severely affected organ after the intestine being the gallbladder, which was largely necrotic. Affected arterioles were also noted microscopically in the pancreas, adrenal capsule, celiac plexus, spleen and kidney. Bilateral chronic pyelonephritis was also present, presumably of long duration, with subtotal atrophy of one kidney and hypertrophy of the other, with both exhibiting acute suppurative exacerbation. The brain could not be examined.

I must confess that at that time no especial significance was attached to the serum as a factor in the vascular lesion. To be sure the clinical diagnosis of periarteritis nodosa was based on the concept that it may represent in some instances disseminated sensitization of blood vessels, probably to bacterial products of focal origin, and as such may have certain analogies with glomerulonephritis, erythema nodosum and possibly rheumatic fever. In her recent paper on clinical aspects of periarteritis nodosa Spiegel (*Arch. Int. Med.* **58**:993, 1936) cited this case among a number of others in which the vascular lesion was viewed as part of a profound systemic response to infection or even to allergic sensitization, e. g., asthma. Since infection was also present in the cases just presented, I wonder whether it might not be helpful to study this problem further in relation to uncomplicated serum sickness, for instance, that resulting from the injection of serum purely for prophylactic purposes, in the absence of infection.

S. H. POLAYES: One of the outstanding lesions of this character is periarteritis nodosa, and every one knows the greater frequency of this disease in males. I wonder whether Dr. Clark has considered sex a factor in sensitivity, and whether, therefore, he has found a greater frequency of sensitiveness of reaction in the male.

HARRY VESELL: Has Dr. Clark any information as to how soon after the injection of serum some of these changes take place? I ask the question because I have in mind a case which I observed several years ago. The operator of a moving picture machine after sustaining a moderate injury to his hand received some antitetanus serum. Seven days later a serum rash developed, and the following day typical angina pectoris. There seems to be a morphologic basis for this condition if changes can take place within a short period. I was wondering whether Dr. Clark has, either in his own experience or in the experiments of the German authors whom he quoted, any evidence as to how soon these morphologic changes take place.

EUGENE CLARK: In reply to Dr. Plaut's question concerning the diffuseness of the vascular reaction described, it may be stated that not all of the coronary vessels show this alteration. Many of them do, but others do not. Concerning the involvement of a single vessel, we cannot say whether the process was focal or diffuse, because serial sections were not done. It is felt that proliferation of the subendothelial mesenchymal tissue in the mural and valvular endocardium is an alteration frequently encountered in much lesser degree. We have seen it in septicemia of various types: gonococcic septicemia, staphylococcic septicemia and streptococcic septicemia. We have not seen it in cases in which an infection of the blood stream was demonstrably absent.

Concerning the question of the predisposition to the development of this lesion related to sex, I am afraid we can supply little information. As yet we have studied at necropsy only seven cases in which serum disease occurred, and of these only five presented material suitable for analysis. Four occurred in males and one in a female, and no significant alterations were observed in the latter. I believe that the limitations of the material do not permit any expression of opinion concerning this point.

Similarly, our experience does not permit any statement concerning the length of time between the administration of serum and the development of these lesions. One subject exhibiting this lesion came to necropsy ten days after the initial administration of serum, and the other died thirty days after the first injection of serum. I suspect that the appearance of these lesions may depend on the intensity of the allergic response in the patient. In experimental animals the latent period apparently varies, though I do not believe any such lesion has been observed within a period of less than seven days after the initial dose of serum.

TUBERCULOUS MENINGITIS IN RELATION TO TUBERCULOMA. DAVID BERES (by invitation) and THEODORE MELTZER (by invitation).

Thirty brains affected with tuberculosis were studied in an attempt to determine what relationship existed between tuberculous meningitis and tuberculous foci in the cerebral substance. The brains were cut coronally by the guillotine method, which yielded over 40 sections per brain. Those which showed no tubercles were resectioned, so that many brains were cut finally into from 120 to 150 sections. The sections were carefully examined for tubercles and other lesions. Routine sections were taken from the interpeduncular space, the ependyma and the choroid plexus. The results were as follows:

1. In a large proportion (fourteen brains) there was diffuse exudative tuberculous meningo-encephalitis without the presense of tubercles in the cerebral substance. As part of the inflammatory process there were, in the cortex, small foci of perivascular infiltration, lymphocytic accumulations and necrobiosis. These changes were in relation to the blood vessels.

2. A small proportion (three brains) showed, in addition to the diffuse tuberculous meningo-encephalitis, a few solitary tubercles in the cerebral substance, which were not in contact with either the ventricular lining or the leptomeninges. It is unlikely that they could have served as foci from which the meningitis developed.

3. In a small quota (six brains) there were, in addition to the tuberculous meningo-encephalitic process, tuberculous lesions in the cerebral substance which were in contact with the leptomeninges. The lesions in four of these brains were small tubercles. These gave the impression of having originated in the depths of a sulcus and then spread to involve the cortex. The remaining two brains in this group revealed large tuberculomas giving clinical manifestations of a cerebral neoplasm.

4. In another group (five brains) there were numerous solitary cortical and subcortical tubercles in the cerebral substance. These were present along with the diffuse tuberculous meningoencephalitis. The great number of tubercles suggests that they were the result of direct dissemination by the blood stream.

5. In two brains tuberculomas in contact with the ventricular lining or with the leptomeninges were present without concurrent diffuse exudative meningitis.

The evidence presented showed that in all cases of tuberculous meningitis there was an extension of the inflammatory process into the cortex, resulting in foci of encephalitis, which varied in degree from perivascular infiltration to tubercle formation. In only six of the thirty brains were cortical tubercles demonstrated which might have been responsible for the coexistent meningitis. In eleven brains the choroid plexus disclosed tubercle formation. The changes in blood vessels which were observed during the study revealed nothing which differed from the

findings already described by other observers. The work was performed under the supervision of Dr. Joseph H. Globus.

DISCUSSION

N. CHANDLER FOOT: The authors have kindly asked me to open the discussion of this paper. In order to appreciate it thoroughly, one must have read at least the last article by Rich and McCordock. This is so complete, so convincing, that it will come as a definite stimulation, if not shock, to any one reading it. They presented a series of ninety odd cases; they went over all of the cases most carefully, employing transillumination on their sections, and came to the conclusion which Dr. Meltzer has given—that the spread of tuberculosis to the meninges was a mediate, not an immediate, affair; that is, the tubercle bacilli had to travel by the blood stream to the brain and set up a focus where they could multiply, and from that focus the bacilli spread to the meninges. They took up the old theory of morphologic evidence of spread from the vessels and decided that this might work just as well were the invasion of the bacilli from the outside of the vessel toward the lumen. They followed up their morphologic examination of the cases by experimenting on a number of guinea-pigs, using a bovine strain. They were unable to produce tuberculous leptomeningitis by intravascular inoculation. They also worked on the possible bearing of allergy on this, and sensitized their animals first, inoculating them later. Here again they were unable to produce typical miliary tuberculous meningitis, but they were able to produce it when they injected the bacteria through the optic foramen. In other words, they did a complete piece of work. Observers have gone for a great many years on the assumption that tuberculous meningo-encephalitis is a blood-borne and blood-distributed infection. It will be found that this is the accepted theory in the textbooks, and therefore Rich and McCordock were flying in the face of tradition. For this reason I think the onus of proving their case is definitely on them.

Here one has an equally careful piece of work, a very careful examination; one has seen how thin the brains were cut and how thoroughly the brains were examined, and the evidence falls on the side of the older school of thought, so that it seems to me that either Rich and McCordock had an unusual number of cases which fell into group III of the present authors or the whole problem will have to be investigated further.

AMOUR F. LIBER: In 1934, in collaboration with Laignel-Lavastine in Paris, I studied three cases of tuberculous meningitis in adults. In all of these cases the condition fell under the section of diffuse exudative meningitis of Beres and Meltzer. This study was published in *Encéphale* in 1935. We paid particular attention to the cellular character of the exudate in the meningeal spaces. In two of our cases there were several tubercles which were subcortical, and, as in the case presented by Drs. Beres and Meltzer, they were chiefly between the cortex and the white matter. We also paid particular attention to the relation between the bacteria, which were shown very readily on bacterial staining, and the meningeal cells. We considered the theories of Rich and McCordock and found no definite evidence for or against them. We concluded that there were fairly characteristic groups of cells in the meningeal exudate, some of which seemed to be derived from the mobilized arachnoid and subarachnoid covering cells. These appeared in various forms, swelled and became vacuolated. Other groups of cells were of an origin more difficult to define, but in any case the grouping of cells in the meninges was always very different from the typical follicular tuberculous grouping in other parts of the body and consisted of diffuse sheets of cells with a tendency to caseous necrosis, but never with giant cells, and at times cells called "epithelioid," presumably of macrophage origin. It is possible to follow cells of this type along perivascular spaces rather deep into the cortex, to a point at which the perivascular space narrowed, beyond which they could not be found. In one fortunate series of sections which went through a cortical tubercle it was possible to follow the meningeal cells in perivascular spaces to the capsule of the tubercle, which was partially made up of these cells. Tubercle bacilli could be demonstrated

in very large numbers in the perivascular spaces, which were filled with cells of the meningeal type. There were many less tubercle bacilli, but they could be found in the remainder of the capsule, which seemed to come from the adventitia of small blood vessels in the cerebral parenchyma. We had the opportunity of studying only three cases, and we could not make as complete serial sections as Drs. Beres and Meltzer have done. However, I believe by following the same procedure and studying the types of cells and the presence of bacteria by bacterial stains one should be able to find information to solve the problem which Rich and McCordock presented. So far, from our studies and those of Drs. Beres and Meltzer, we have not been able to draw any conclusion. I think we need a different method of attack, and cytologic and histobacteriologic methods should be of assistance. My impression from the small number of cases which I have studied is that both mechanisms are possible, and in at least one of the cases which I mentioned it seems possible that the tubercle in the cortex was a lesion which had spread by contiguity from the subarachnoid space through the perivascular funnel. Dr. Walter Freeman, of Washington, D. C., made a suggestion which is valuable, namely, that there may be a tendency of all the cells belonging to the histologic class of subarachnoid covering cells to react in this peculiar way, and that would include cells lining the perivascular spaces.

In any case, I think that Drs. Beres and Meltzer are to be congratulated on their industry in gathering such a large amount of material and in analyzing it so clearly. I should like to ask whether they made bacterial stains and studied the morphologic relation of the bacteria to the exudate.

S. H. POLAYES: Were there any foci which might be considered primary elsewhere in the body in the cases studied?

THEODORE MELTZER: As regards the first question, that of the relationship between the bacteria and the tubercle, the only bacteriologic investigations we have made consisted in confirming the tuberculous nature of the process. No cultures were made. The brains, of course, were already fixed, and therefore no attempt was made to correlate the type of bacteria with the tuberculous foci.

In reply to the second question, that of the primary foci, that matter was traced in a large number of cases. We had the postmortem protocols in a hundred instances of tuberculous meningitis in which there had been full postmortem examination, and in 90 per cent of the cases primary foci were found somewhere in the body. In 10 per cent of the cases no primary foci were found.

I should like to say also that Dr. Beres and I are in agreement that in certain instances tuberculous meningitis may result from a tuberculous focus in the cerebral substance, but to say that is the cause in all cases is not in accordance with what Ragins of Chicago has found, or with what Dr. Liber, who studied the three cases in Salpêtrière, has found, or with the results of the study presented this evening.

Book Reviews

Christian R. Holmes, Man and Physician. By Martin Fischer. Cloth. Price, $4. Pp. 233, with illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

This biography of Cincinnati's indomitable, far seeing medical leader in the early years of the present century is from the pen of versatile Martin Henry Fischer. For twenty-seven years the author has been professor and head of the department of physiology in the college of medicine at the University of Cincinnati. He has been a forceful member of the faculty, a popular and stimulating teacher. Recipient of national and international honors for his work and contributions in the field of colloid chemistry, he uses a palet, writes of painting and translates the original works of ancient sages with equal facility.

"Life has been compared to chess. Its men have qualities; and move under rules. In the following pages I am the recorder of a game. I knew Christian R. Holmes as a king amid smaller pieces and pawns. What is said is recitation of why he was not checkmated."

After this preface the author briefly traces the life of his subject, in three short chapters, from birth through youth, education and marriage to the beginning of his public career and his leadership in the medical affairs of Cincinnati.

Then, in succeeding chapters, there is related in considerable detail the gradual development, through the vagaries of civic and medical politics, of the plans and the construction of the new college building, the development of closer affiliations between college and hospital, whereby a large measure of university control is effected in the latter institution, and the efforts to raise the standards of medical education. Holmes' plans whereby he hoped to create a fine medical center in the Ohio Valley are disclosed. The measure of his success is evidenced by the splendid institutions that stand as memorials to his labors.

The concluding chapters relate the active rôles taken by Dr. and Mrs. Holmes and their sons in the World War and the renewal of his work at college and hospital, which was so shortly thereafter terminated by his untimely death.

The author has an interesting style of expression, one which holds the attention of the reader. The narrative, while not free from factual errors, is a delineation in colors which reflect the biographer's intimate associations with civic affairs and with Cincinnati's great leader and protagonist for the proper development of medical education and hospital service.

The volume is an excellent example of the bookmaker's art. Sententious maxims which mark particular events as marginal notes on numerous pages add much of interest.

This biography will be welcomed not only by those who knew Christian R. Holmes but by those who are interested in the development of medical education and in its progress in the last several decades.

Clio medica: Pathology. By E. B. Krumbhaar, Professor of Pathology, University of Pennsylvania School of Medicine. Cloth. Price, $2. Pp. 206, with 18 illustrations. New York: Paul B. Hoeber, Inc., 1937.

"Clio medica" is the general title of a series of primers on the history of medicine edited by the author of this little book, which is the nineteenth in the series. The aim of the series is to present "in a concise and readable form a number of special phases of the long and complex history that underlies the great edifice of modern medical science." The book does not review the whole history of pathology in the broader sense. Its chapters deal with the following topics: primitive, classical and medieval conceptions of disease; the rise of the anatomic idea in disease; systematized gross pathologic anatomy; pathologic anatomy of the tissues and precellular pathology; cellular pathology; integrated pathology

(structural, functional, chemical, experimental and clinical methods of approach); inflammation and cancer. The history of infection does not receive systematic consideration. There are brief appendixes on the word pathology and on early chairs of pathology and pathologic anatomy. There is also a chronological list of pathologic milestones which takes a wider field than the text but "makes no pretense at completeness or complete accuracy." Ricketts' work on Rocky Mountain spotted fever should have been included; also Schenck's discovery of sporotrichosis in 1900. There are 18 illustrations, nearly all noteworthy portraits of great leaders. Krumbhaar's book gives a scholarly, instructive and readable account of the development of pathology. It merits reading by any one who is interested in the growth of the knowledge of disease.

L'hormone folliculaire en physiologie normale et pathologique; étude expérimentale clinique et thérapeutique. By Dr. Henri Simonnet, professeur a l'École nationale vétérinaire d'Alfort, chef du laboratoire de physiologie du Centre de prophylaxie mentale de la Seine (Hôpital Henri-Rousselle). Price, 100 francs. Pp. 531. Paris: Masson & Cie, 1937.

This volume is a review, encyclopedic in scope, of the present state of knowledge concerning the estrogenic hormone. The text is exceptionally well documented with 2,500 references to the international scientific and medical literature, which probably constituted a complete bibliography on this subject at the time of writing. Indeed, the final 181 pages, containing the bibliography and the subject and author indexes, have permanent value quite independent of any future obsolescence of the textual matter.

The subject matter is systematically arranged in short sections, marked by subheadings. The exposition of the material on each topic is well balanced and concise and includes clinical and therapeutic as well as physiologic considerations. The author has usually included a brief interpretative summary statement, in a pleasingly unobtrusive manner.

The work should prove valuable to the research worker in this field of endocrinology and is an excellent book of reference for the medical student or physician seeking detailed information on a particular phase of estrogenic activity. The author is to be congratulated on the creditable accomplishment of a difficult and useful task.

Précis de médecine coloniale. By Ch. Joyeux, professeur de parasitologie à la Faculté de médecine de Marseille, ex-médecin colonial, and A. Sicé, professeur à l'École d'application du corps de Santé colonial de Marseille. Second edition. Cloth. Price, 170 francs. Pp. 1,250, with 240 illustrations. Paris: Masson & Cie, 1937.

This is the second edition of a useful book, which is intended especially for physicians who practice in warm countries as well as for those preparing for such practice. To facilitate presentation, the book is divided into three parts, dealing with (1) the diseases of organs, (2) febrile diseases and (3) general diseases. In the accounts of the individual diseases the following plan of topics is followed: historical definition, geographic distribution, epidemiology, symptoms, complications, structural changes, diagnosis, prognosis, treatment, prevention. The consideration is not limited to diseases peculiar to the tropics but includes diseases occurring also elsewhere which show peculiarities in tropical regions. The illustrations, while not elegant, are instructive. The book is well written and comprehensive, yet compact.

Books Received

LES IMMUNITÉS LOCALES. A. Besredka, professeur a l'Institut Pasteur. Paper. Price, 35 francs. Pp. 224. Paris: Masson & Cie, 1937.

SOME QUANTITATIVE ASPECTS OF THE BIOLOGICAL ACTION OF *X* AND *Y* RAYS. C. M. Scott. Medical Research Council, Special Report Series, no. 223. Paper. Price, 1 shilling, sixpence. Pp. 99. London: His Majesty's Stationery Office 1937.

ATLAS OF HEMATOLOGY. Edwin E. Osgood, M.A., M.D., Assistant Professor of Medicine and Head of Experimental Medicine, University of Oregon Medical School, Portland, Ore., and Clarice M. Ashworth, Medical Illustrator, University of Oregon Medical School, Portland, Ore. Cloth. Price, $10. Pp. 255, with 326 illustrations in color. San Francisco: J. W. Stacey, 1937.

MECANISMO PROBABLE DE LA CANCERIZACION (ENSAYO PATOGENICO). Americo Garibaldo, profesor de la Facultad de Ciencias Médicas, Universidad Mayor de San Marcos, Lima, Peru. 2 volumes. Paper. Lima, Peru: Facultad de Ciencias Médicas, Universidad Mayor de San Marcos, 1936.

PATHOLOGY OF THE CENTRAL NERVOUS SYSTEM. A STUDY BASED UPON A SURVEY OF LESIONS FOUND IN A SERIES OF FIFTEEN THOUSAND AUTOPSIES. Cyril B. Courville, M.D., Professor of Neurology and Psychiatry, College of Medical Evangelists, and Director, Cajal Laboratory of Neuropathology, Los Angeles County Hospital, Los Angeles, Calif. Cloth. Price, $5.75. Pp. 344, with 200 illustrations. Mountain View, Calif.: Pacific Press Publishing Association, 1937.

THE PATIENT AND THE WEATHER. William F. Petersen, M.D., with the assistance of Margaret E. Milliken, S.M. Volume IV, part 2. ORGANIC DISEASE: HYPO AND HYPERTHYROIDISM, DIABETES, THE BLOOD DYSCRASIAS, TUBERCULOSIS. Cloth. Price, $11. Pp. 729, with 380 illustrations. Ann Arbor, Mich.: Edwards Brothers, Inc., 1937.